T H E S I S

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DEGREE OF DOCTOR OF PHILOSOPHY

by

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A C K N O W L E D G E M E N T S

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The Structure of Mycophenolic Acid.

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PART I.

THE STRUCTURE OF MYCOPHENOLIC ACID.

SUMMARY

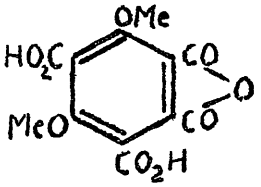
The constitution of mycophenolic acid, a mould metabolite of *P. brevi-compactum*, is discussed and its structure confirmed as 4-methyl-5-methoxy-6-(5'-carboxy-3'-methylpent-2'-enyl)-7-hydroxyphthalide by the synthesis of 4-methyl-5:7-dimethoxy-6-carboxymethylphthalide, a degradation product of the monomethyl ether of the acid and by infrared spectroscopy of the acid and its methyl ether.

INTRODUCTION

Gosio[1] isolated from mouldy Italian maize a strain of Penicillium, now known to be P. brevi-compactum, which, when grown on maize itself or on Raulin's medium, produced a metabolite which gave an intense blue colour with ferric chloride. Alsberg and Black[2] isolated the mould P. stoloniferum Thom from a source similar to that of Gosio and when grown on Raulin's medium the mould produced a metabolite, mycophenolic acid, giving a blue colouration with ferric chloride. Clutterbuck, Oxford, Raistrick and Smith[3] regard Gosio's acid as identical with mycophenolic acid and Thom[4] regards P. brevi-compactum and P. stoloniferum as synonyms.

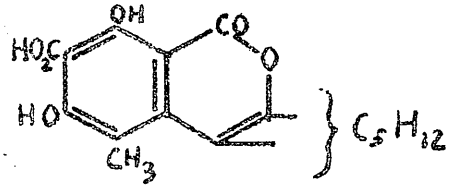
Mycophenolic acid, m.p. 141°, together with other metabolic products, has been isolated from twelve strains of P. brevi-compactum by Clutterbuck, et al.[3], in yields of 50 to 420 mg. per litre of culture medium. It had a molecular formula of $C_{17}H_{20}O_6$ and had one methoxyl group. The acid readily underwent catalytic hydrogenation, taking up one mole of hydrogen per mole of acid. Clutterbuck and Raistrick[5] showed that mycophenolic acid contained a lactone ring, that it formed a mono-acetate and a monomethyl ether which gave no colour with ferric chloride and that it was demethylated to normycophenolic acid. Oxidation of mycophenolic acid methyl ether with alkaline potassium permanganate first attacked the double bond and ultimately produced a tetracarboxylic acid

which analysed as the anhydride (I) of 1:5-dimethoxybenzene--2:3:4:6-tetracarboxylic acid.



(I)

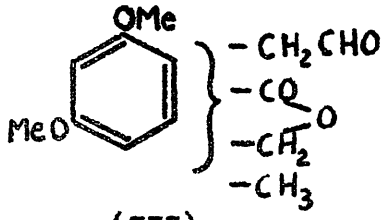
Fusion of mycophenolic acid with potassium hydroxide produced 2-methylorcinol and on the basis of these facts the tentative structure (II) for normycophenolic acid was advanced.



(II)

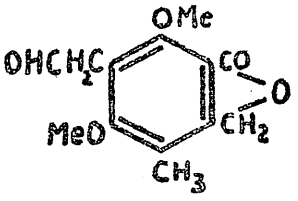
Birkinshaw, Bracken, Morgan and Raistrick[6] observed that ozonolysis of mycophenolic acid and its monomethyl ether produced in each case levulic acid, the other products being aldehydes of molecular formula $C_{12}H_{12}O_5$ and $C_{13}H_{14}O_5$ respectively. A small amount of the corresponding acid of molecular formula $C_{13}H_{14}O_6$ was also formed in the case of the methyl ether. The C_{13} aldehyde formed an enol-acetate which underwent ozonolysis to give an aldehyde of molecular formula $C_{12}H_{12}O_5$. The C_{13} aldehyde was neutral but titrated hot as a mono-basic acid while the corresponding C_{13} acid, obtained from the aldehyde by alkaline iodine oxidation, titrated cold as a mono-basic and hot as a dibasic acid. The constitution of the C_{13} aldehyde

was therefore given as (III), and having regard to the



(III)

production of the anhydride (I), the four possible isomers were as follows:-



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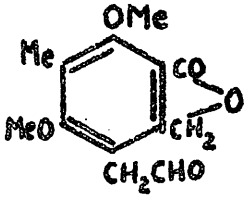

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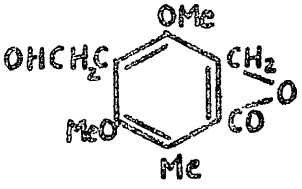
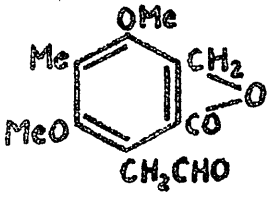
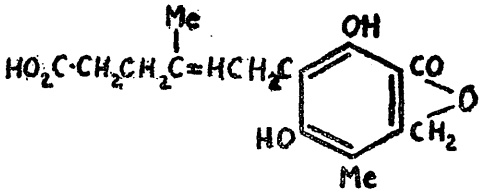


(VI)

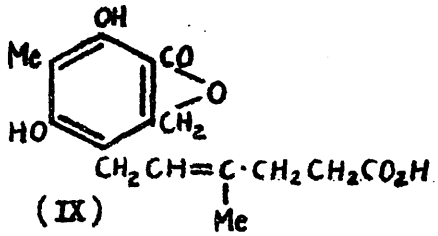


(VII)

A positive Gibbs test[7] on normycophenolic acid, the blue ferric chloride colour of mycophenolic acid and the production of 2-methylorcinol by potassium hydroxide fusion eliminated structures (VI) and (VII) for the C_{13} aldehyde and the two most probable formulae for normycophenolic acid became (VIII) and (IX).

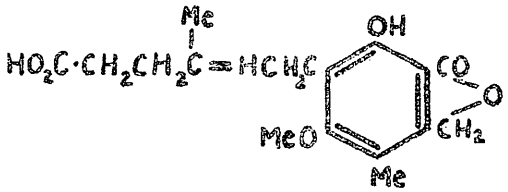


(VIII)

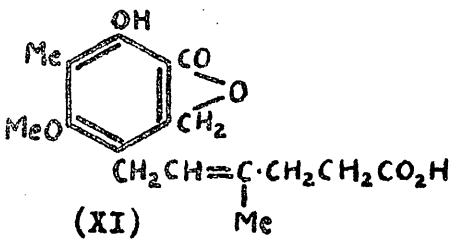


(IX)

By virtue of a negative Gibbs test on mycophenolic acid and the supporting evidence of the blue ferric chloride colour, the hydroxyl group para to the potential carboxyl group in (VIII) and (IX) appeared to be the one which was methylated in mycophenolic acid thus giving (X) and (XI) as the most probable structures for mycophenolic acid.



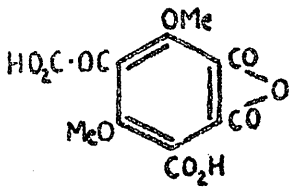
(X)



(XI)

Birkinshaw, Raistrick, Ross and Stickings[8], in the course of work on cyclopolic acid, found that hot alkaline permanganate oxidation of methyl cyclopolate produced a tetracarboxylic acid, analysed as its anhydride, which was beyond doubt 4:6-dimethoxybenzene-1:2:3:5-tetracarboxylic acid. Birkinshaw, Raistrick and Ross[9] showed that this anhydride depressed the melting point of the anhydride reported by Clutterbuck and Raistrick[6] as the oxidation product of mycophenolic acid methyl ether. A careful re-examination by Birkinshaw, et al.[9], of the oxidation of

mycophenolic acid methyl ether showed the analytical data of the oxidation product to be more in keeping with a molecular formula of $C_{13}H_8O_{10}$ than with that of $C_{12}H_8O_9$ originally assigned. This C_{13} oxidation product was established as (XII), further oxidation of its sodium salt with hydrogen peroxide at 0° giving the anhydride of 4:6-dimethoxybenzene-1:2:3:5--tetracarboxylic acid.



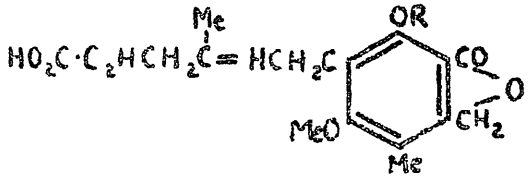
(XII)

The $-CO.CO_2H$ group thus demonstrated the site of the $-CH_2.CH{=}C(CH_3).CH_2.CH_2.CO_2H$ side-chain in mycophenolic acid and eliminated (XI) as a possible structure. Structure (X) therefore remained as almost certainly the correct one for mycophenolic acid.

The antibiotic nature of mycophenolic acid was demonstrated by Gosio[1] who showed that it suppressed the growth of the anthrax bacillus and it appears probable that mycophenolic acid was the first crystalline antibiotic of fungal origin to be isolated. Its antibacterial action against several animal and plant pathogens and also against a number of saprophytic and pathogenic fungi has been evaluated by Florey and his co-workers[10] who have shown it to be more effective against Gram positive than against Gram negative bacteria.

THEORETICAL

The structure of mycophenolic acid has been shown by the degradative studies of Raistrick and his co-workers[3,5,6,9] to be almost certainly structure (XIII, R = H) although Birkinshaw, Raistrick and Ross[9] admitted slight doubts as to which of the two nuclear hydroxyl groups in normycophenolic acid was methylated in mycophenolic acid and as to whether the potential hydroxymethyl group in the phthalide ring was <u>ortho</u> or <u>meta</u> to the nuclear methyl group.

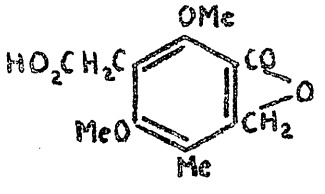


(XIII)

The evidence for assigning the 5-hydroxyl group in normycophenolic acid as the one which is methylated in mycophenolic acid has been from colour tests alone and it was felt that infrared spectroscopy could provide certain confirmation of this assignment. Duncanson, Grove and Zealley[11] have reported that the infrared absorption spectra of 7-hydroxyphthalides in dilute solution show, due to chelation, a marked decrease in the frequencies of the phthalide carbonyl stretching vibration compared with those of the corresponding methyl ethers. A decrease in the phthalide carbonyl stretching frequency in mycophenolic acid compared with that of its methyl ether (XIII, R = Me) would therefore indicate the presence in

mycophenolic acid of an o-hydroxyaroyl system. The infrared spectra of mycophenolic acid show phthalide carbonyl stretching frequency maxima at 1742 ($CHCl_3$), 1748 (CCl_4) and 1751 $cm.^{-1}$ (dioxan) while the methyl ether shows maxima at 1763, 1776 and 1770 $cm.^{-1}$ in the same solvents, respectively. These results clearly indicate the presence of intramolecular hydrogen bonding in mycophenolic acid and confirm the position of the free hydroxyl group to be that given in structure (XIII, R = H).

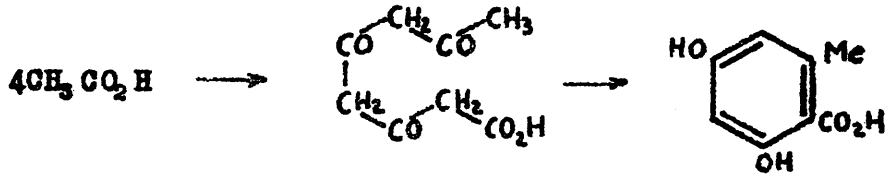
In order to obtain a clear decision on the orientation of the potential hydroxymethyl group in mycophenolic acid methyl ether (XIII, R = Me), it was aimed to synthesise 6-carboxymethyl-5:7-dimethoxy-4-methylphthalide (XIV). This acid has been obtained[6] as the minor product of ozonolysis of mycophenolic



(XIV)

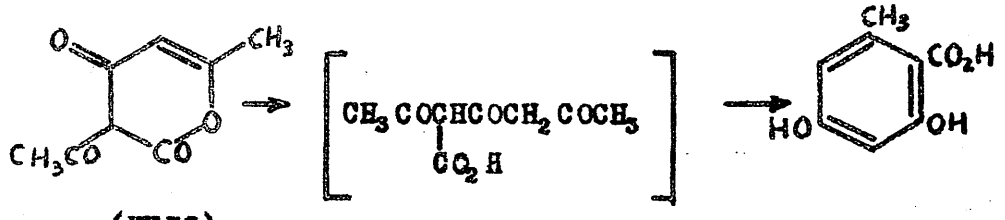
acid methyl ether or by alkaline iodine oxidation of the corresponding aldehyde (obtained as the major product of ozonolysis). Aghoramurthy and Seshadri[12] have proposed a theory of biosynthesis of mould products in which orsellinic acid (XV) is advanced as the fundamental unit. The biosynthesis of orsellinic acid in the first place can be explained by the acetate hypothesis of Birch and Donovan[13,14]. Head-to-

-tail linkage of four acetic acid molecules would give the polyketone (XVI) which would then cyclise by an aldol-type condensation to give orsellinic acid.



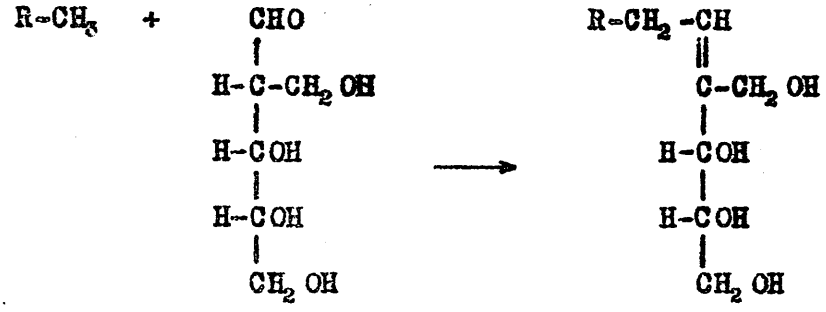
(XVI) (XV)

A laboratory preparation of orsellinic acid by a similar type of condensation has been reported[13,15] starting from dehydracetic acid (XVII).



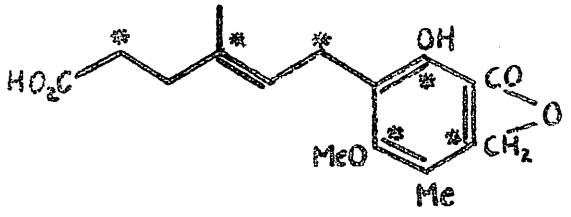
(XVII)

By means of nuclear and O-methylation and side-chain oxidation, it can be readily seen how the biosynthesis of mycophenolic acid from orsellinic acid is effected. Aghoramurthy and Seshadri[12] attributed the side-chain lengthening in the 6-position to condensation with a branched-chain hexose (XVIII) in the manner shown.



(XVIII)

Recent work by Birch and his co-workers[16] has shown that acetic acid labelled at the carboxyl carbon atom is incorporated into both the nucleus and the C_7 side-chain of mycophenolic acid as shown in the labelling pattern (XIX; labelled atoms = *), while the nuclear and O-methyl groups are supplied by methionine.



(XIX)

The terpenoid origin of the C_7 side-chain has been demonstrated by the incorporation of [α - ^{14}C] mevalonic acid (β δ -dihydroxy--β-methylvaleric acid)[17] to give mycophenolic acid in which all the activity is present in the levulic acid obtained by ozonolysis, the nucleus being unlabelled.

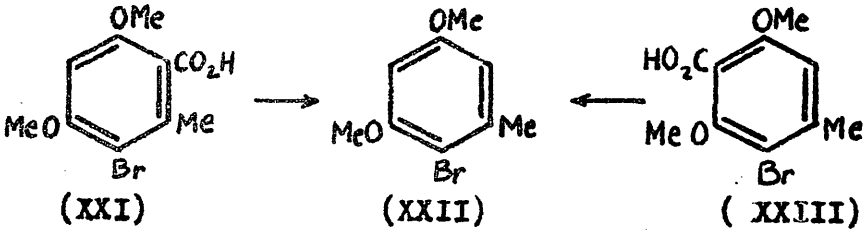
Since there existed efficient and convenient laboratory methods for reproducing these biogenetic methylations and oxidations, orsellinic acid dimethyl ether (XX) was chosen as the starting material for the synthesis of the acid (XIV).

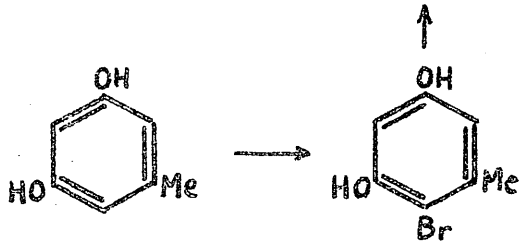
The first step in the synthesis was the formation of the lactone by side-chain oxidation of the ortho-methyl group in the acid (XX) to hydroxymethyl with subsequent ring closure. This was effected by the photobromination technique.[18] Treatment of (XX) with 1½ mol. of bromine using this technique gave the expected 3-bromo-4:6-dimethoxy-2-methylbenzoic acid (XXI).

(XX) (XXI)

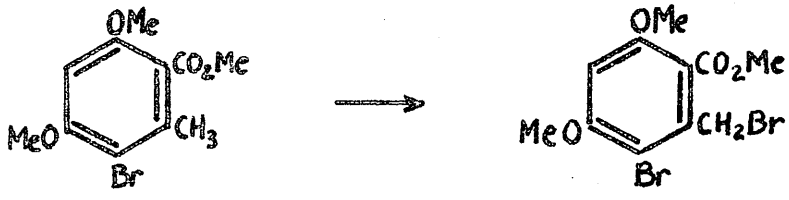
The orientation of the bromine atom in (XXI) was established by decarboxylation to 2-bromo-3:5-dimethoxytoluene (XXII) which has been prepared by decarboxylation[19] of 3-bromo-2:6--dimethoxy-4-methylbenzoic acid (XXIII). The decarboxylation product was found to be identical with a specimen of 2-bromo--3:5-dimethoxytoluene prepared by bromination of orcinol (XXIV) followed by methylation.[20] When photobromination was carried out with 2 mols. of bromine and followed by alkaline hydrolysis, orsellinic acid dimethyl ether gave 4-bromo-5:7--dimethoxyphthalide (XXV) as the major product, together with some of the bromo-acid (XXI). In order to establish the bromo-acid (XXI) as an intermediate in the preparation of

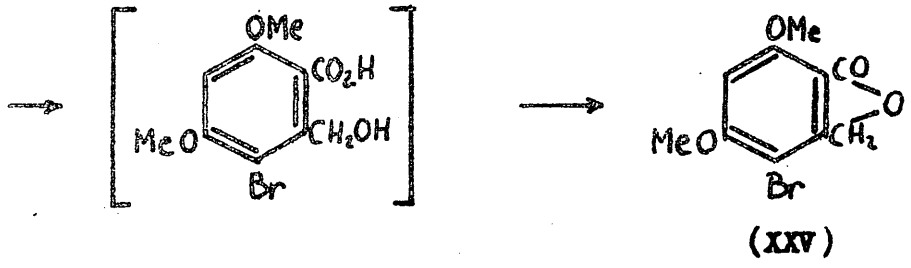
the phthalide (XXV), the acid was esterified (in order to render it soluble in non-polar solvents) and the ester (XXVI) photo-brominated with 1 mol. of bromine to give methyl 3-bromo-2--bromomethyl-4:6-dimethoxybenzoate (XXVII). The latter





compound underwent alkaline hydrolysis to form the hydroxymethyl acid which lactonised yielding 4-bromo-5:7-dimethoxyphthalide (XXV).



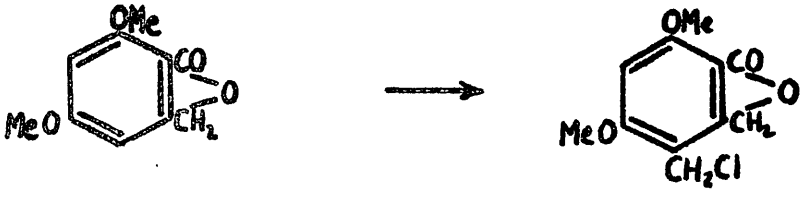


The phthalide (XXV) was also prepared by treating orsellinic acid dimethyl ether with 1 mol. of N-bromosuccinimide to give the acid (XXI) which was methylated and treated with a further mol. of the reagent followed by hydrolysis. These experiments show that the first step in the photobromination of orsellinic acid dimethyl ether with bromine or N-bromosuccinimide is the nuclear substitution of a bromine atom in the 3-position and that this ortho-bromo group is necessary to activate the methyl group for subsequent side-chain bromination. Catalytic hydrogenolysis of the phthalide (XXV) yielded 5:7-dimethoxyphthalide (XXVIII) which underwent alkaline permanganate oxidation to the known 3:5-dimethoxyphthalic acid.[21]

The next step in the synthesis was the nuclear methylation of the phthalide (XXVIII) in the 4-position and this was effected by chloromethylation with formaldehyde and hydrochloric acid followed by reductive removal of the chlorine atom. The two possible products of monochloromethylation were 4-chloromethyl-5:7-dimethoxyphthalide (XXIX) or the 6-chloromethyl isomer and using a modification of the mild conditions of Wilson, et al.,[22] a monochloromethyl derivative was formed which was proved to be the 4-substituted phthalide (XXIX) by the fact that on hydrolysis with aqueous sodium carbonate, the resultant hydroxymethyl compound (XXX) underwent rearrangement† to 5:7-dimethoxyphthalan-4-carboxylic acid (XXXI) on treatment with

† This rearrangement is discussed in detail in Part II of this Thesis.

methanolic sodium methoxide. A small proportion of the phthalan carboxylic acid was also produced during the carbonate hydrolysis of the chloromethyl compound (XXIX).



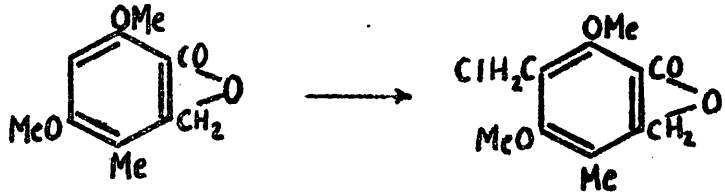
(XXVIII) (XXIX)

(XXX) (XXXI)

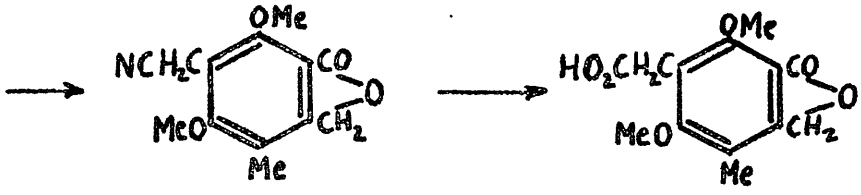
Catalytic hydrogenolysis of (XXIX) gave 5:7-dimethoxy-4--methylphthalide (XXXII).

The final step in the synthesis of the acid (XIV) was the introduction of the carboxymethyl group into the remaining vacant 6-position in the phthalide (XXXII). This was achieved by chloromethylation followed by side-chain lengthening with potassium cyanide. Vigorous treatment of the phthalide (XXXII) with formaldehyde and hydrochloric acid gave 6-chloromethyl-5:7-dimethoxy-4-methylphthalide (XXXIII) which readily reacted with potassium cyanide to give 6-cyano-methyl-5:7-dimethoxy-4-methylphthalide (XXXIV). Hydrolysis of the latter compound with aqueous potassium hydroxide yielded 6-carboxymethyl-5:7-dimethoxy-4-methylphthalide (XIV), which was found to be identical with a sample

of the degradation acid prepared by ozonolysis of mycophenolic acid methyl ether and by alkaline iodine oxidation of the corresponding aldehyde, the melting point of the two acids being undepressed on mixing and their infrared absorption spectra being identical.



(XXXII) (XXXIII)

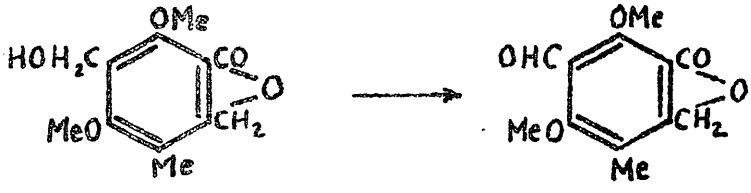


(XXXIV) (XIV)

In addition, the methyl esters of the synthetic and degradation acids were prepared and showed no depression of melting point on admixture. The unambiguous synthesis of 6-carboxymethyl-5:7-dimethoxy-4-methylphthalide thus positively confirms the orientation of the lactone ring in mycophenolic acid methyl ether as being that shown in structure (XIII, R = Me) and consequently the structure of mycophenolic acid is confirmed as structure (XIII, R = H).

As additional confirmation of structure (XIII, R = Me), 6-chloromethyl-5:7-dimethoxy-4-methylphthalide (XXXIII) was hydrolysed with aqueous carbonate to 6-hydroxymethyl-5:7-

-dimethoxy-4-methylphthalide (XXXV) which underwent chromic acid oxidation to give 6-formyl-5:7-dimethoxy-4-methylphthalide (XXXVI) m.p. 131.5-133°; 2:4-dinitrophenylhydrazone m.p. 226° (decompn.). This aldehyde has been reported by Birkinshaw et al.[6], as the end-product of side-chain degradation of mycophenolic acid methyl ether and while it has not been possible to make a direct comparison between the synthetic aldehyde and a crystalline specimen of that, m.p. 125-126° with 2:4-dinitrophenylhydrazone m.p. 228-230° (decompn.), obtained by degradation, we feel that the latter authors' aldehyde was incompletely purified.



(XXXV) (XXXVI)

The same conclusion regarding the structure of mycophenolic acid has been reached independently by Professor A. J. Birch,[23] whose helpful co-operation we acknowledge.

EXPERIMENTAL

All melting points are uncorrected. Ultraviolet absorption spectra were determined in ethanol solution.

Mycophenolic Acid. - The mould Penicillium brevi--compactum was grown on Raulin-Thom medium and mycophenolic acid, m.p. 140-142°, isolated as described by Raistrick and his co-workers[3] (lit. m.p. 141°). The yield of crude acid was 380 mg. per litre of culture medium.

5-Methyl-6-carbethoxycyclohexane-1:3-dione. (cf. Schilling and Vorländer, Annalen, 1898, 308, 195). - To a solution of sodium (20.7 g.) in ethanol (300 c.c.) at room temperature was added ethyl acetoacetate (126 g.) and ethyl crotonate (102 g.). The solution was heated on the steam-bath for 2 hr. and the deposited white crystalline solid collected on cooling. The solid was washed by reslurrying once with ethanol and once with ether, sucked dry and dissolved in cold water (400 c.c.). Acidification (Congo red) of the solution with dilute sulphuric acid precipitated the product as an oil which solidified to a granular solid on stirring. The solid product was collected, washed by reslurrying with water, sucked dry and desiccated. Yield 84.8 g., m.p. 88-90° (lit. m.p. 89-90°).

Ethyl Orsellinate. (cf. St. Pfau, Helv. Chim. Acta, 1933, 16, 282). - 5- Methyl-6-carbethoxycyclohexane-1:3-dione (25 g.)

was dissolved in glacial acetic acid (52.5 c.c.), diluted with water (200 c.c.) and anhydrous ferric chloride (50 g.) added with shaking. The dark brown solution was heated under reflux for 1 hr. and, after standing overnight, diluted with water (250 c.c.) and extracted with ether (3 x 500 c.c.). The combined extract was washed with aqueous sodium hydrogen carbonate (3 x 500 c.c.; 10%), water (500 c.c.) and dried (Na_2SO_4). Evaporation of the ether gave crude ethyl orsellinate (12.9 g.). The crude solid was dissolved in the minimum amount of hot benzene. On cooling, the crystalline product (8.1 g.) was collected and washed by reslurrying with light petroleum (b.p. 60-80°). The m.p. 128-130° (lit. m.p. 131-132°) was undepressed on admixture with an authentic specimen.

Orsellinic Acid Dimethyl Ether. - Since there was no reported method for the preparation of orsellinic acid dimethyl ether from ethyl everninate, the following procedure was adopted. Ethyl everninate (5.84 g.), prepared from ethyl orsellinate by the method of Fischer and Hoesch[24] in 71% yield, was heated on the steam-bath for 1½ hr. with aqueous sodium hydroxide (58 c.c.; 2N). The cooled solution was stirred with dimethyl sulphate (2.5 c.c.). After 1½ hr. dimethyl sulphate (2.5 c.c.) was added and thereafter five additions of dimethyl sulphate (each 2.5 c.c.) together with aqueous sodium hydroxide (each 11 c.c.; 2N) were made at

hourly intervals with continuous stirring. 2 hr. after the last addition, aqueous sodium hydroxide (11 c.c.; 2N) was added and the solution refluxed for 1½ hr. The cooled solution was acidified (Congo red) with hydrochloric acid (d,1.15) and the precipitated orsellinic acid dimethyl ether (5.6 g.) collected. Crystallisation from aqueous ethanol gave plates, m.p. 142-143° (lit.,[28] m.p. 143-144°).

3-Bromo-4:6-dimethoxy-2-methylbenzoic Acid. - (a) A partial solution of orsellinic acid dimethyl ether (1.0 g.) in dry carbon tetrachloride (25 c.c.), heated under reflux and irradiated by a 150 w. lamp, was treated dropwise with bromine (0.527 c.c.; 2 mol.) during 10 min. and refluxing continued for 1½ hr. The solvent was removed under reduced pressure and the solid residue heated under reflux for 3 hr. with aqueous sodium hydroxide (50 c.c.; 2 N). The cooled solution was extracted with chloroform (50 c.c.) and acidified (Congo red) with hydrochloric acid (d,1.15). The precipitate was extracted with chloroform (3 x 50 c.c.) and the extract (A) washed with aqueous sodium hydrogen carbonate (3 x 50 c.c.; 10%). Acidification (Congo red) of the aqueous washings gave 3-bromo-4:6-dimethoxy-2-methylbenzoic acid (0.48 g.) which separated from aqueous ethanol as needles, m.p. 210° (decomp.) (Found: C,43.7; H,4.0%; equivalent, 276. $C_{10}H_{11}O_4Br$ requires C,43.65; H,4.0%; equivalent, 275), light absorption: Max. at 2070

(ε = 34,000) and 2860 Å. (ε = 3,550). The methyl ester, prepared with diazomethane, crystallised from aqueous methanol as needles, m.p. 120-121° (Found: C,45.5; H,4.7. $C_{11}H_{13}O_4Br$ requires C,45.7; H,4.5%), light absorption: Max. at 2065 (ε = 32,000) and 2895 Å. (ε = 3,350). Photobromination of orsellinic acid dimethyl ether (1.0 g.) with bromine (0.395 c.c., 1.5 mol.) gave a higher yield of the bromo-acid (0.74 g.).

(b) (With Dr. John Blair). A solution of orsellinic acid dimethyl ether (300 mg.) in carbon tetrachloride (20 c.c.) and benzene (10 c.c.) was refluxed on the steam-bath with N-bromo-succinimide (1.05 mol.) for 5 hr. with irradiation from a 60 w. lamp. The cooled reaction mixture was evaporated to dryness under reduced pressure and the residual solid shaken with ether and aqueous sodium hydrogen carbonate (10%). Acidification (Congo red) of the aqueous phase with hydrochloric acid and crystallisation of the precipitate from aqueous ethanol gave the bromo-acid (100 mg.), m.p. 210-211° (decomp.) alone or mixed with a sample of preparation (a) (Found: C,43.65; H,4.0%).

Methyl 3-Bromo-2-bromomethyl-4:6-dimethoxybenzoate. - A solution of methyl 3-bromo-4:6-dimethoxy-2-methylbenzoate (250 mg.) in carbon tetrachloride (10 c.c.) was photobrominated as in (a) above with bromine (0.138 g.; 1 mol.). Refluxing was continued for 1 hr. after the addition of bromine had been completed. Removal of the solvent under reduced pressure gave

an oil which rapidly solidified. Crystallisation from light petroleum (b.p. 60-80°) gave methyl 3-bromo-2-bromomethyl--4:6-dimethoxybenzoate (300 mg.) as needles, m.p. 118.5-119° (Found: C,36.3; H,3.5. $C_{11}H_{12}O_4Br_2$ requires C,35.9; H,3.3%), light absorption: Max. at 2200 (ε = 25,000) and 3070 Å. (ε = 3,900). The same compound, m.p. and mixed m.p. 118-119°, was obtained in almost quantitative yield by photobromination of methyl orsellinate dimethyl ether with bromine (2 mol.).

4-Bromo-5:7-dimethoxyphthalide. - (i) Methyl 3-bromo-2--bromomethyl-4:6-dimethoxybenzoate (0.6 g.) was heated under reflux with aqueous sodium hydroxide (25 c.c.; 2N) for 2 hr. The almost complete solution was cooled, extracted once with chloroform (25 c.c.) and the extract rejected. The aqueous phase was acidified (Congo red) with hydrochloric acid (d,1.15) and the resulting precipitate extracted with chloroform (50 c.c.). The extract was washed with aqueous sodium hydrogen carbonate (2 x 25 c.c.; 10%), water and dried (Na_2SO_4). Evaporation of the chloroform under reduced pressure and crystallisation of the residual solid from chloroform-methanol gave 4-bromo-5:7--dimethoxyphthalide (330 mg.) as fine needles, m.p. 246-248° (Found: C,44.5; H,3.5. $C_{10}H_9O_4Br$ requires C,44.0; H,3.3%), light absorption: Max. at 2205 (ε = 35,400), 2590 (ε = 12,400) and 2985 Å. (ε = 5,900). The infrared absorption spectrum of the bromo-phthalide in chloroform solution showed a strong band at 1767 $cm.^{-1}$ characteristic of the phthalide carbonyl stretching

vibration. When the photobromination of orsellinic acid dimethyl ether (1 g.) was carried out with 1½ mol. of bromine, the yield of 4-bromo-5:7-dimethoxyphthalide was 0.29 g. (ii). The chloroform extract A, above, was washed with water, dried (Na_2SO_4) and evaporated under reduced pressure. The residue was hydrolysed with aqueous sodium hydroxide and the bromo--phthalide (0.6 g.) isolated as described in (i). It separated from chloroform-methanol as needles, m.p. and mixed m.p. 246-248° (Found: C,44.2; H,3.4%). (iii) Methyl 3-bromo--4:6-dimethoxy-2-methylbenzoate (250 mg.) in dry carbon tetrachloride (10 c.c.) and dry benzene (10 c.c.) was heated under reflux and irradiated by a 150 w. lamp for 1½ hr. with N-bromosuccinimide (162 mg.; 1.05 mol.). The mixture was cooled, filtered from succinimide and the filtrate evaporated to dryness under reduced pressure. The residual brown oil was heated under reflux for 2 hr. with aqueous sodium hydroxide (20 c.c.; 2N) and the cooled, filtered solution extracted with chloroform. The extract was washed with aqueous sodium hydrogen carbonate (10%), water and dried (Na_2SO_4). Evaporation of the chloroform gave the bromo-phthalide (66 mg.) which crystallised from chloroform-methanol as needles, m.p. and mixed m.p. 243.5-244.5°. On acidification of the sodium hydrogen carbonate washings and extraction with chloroform,

3-bromo-4:6-dimethoxy-2-methylbenzoic acid (129 mg.) was recovered.

Bromoorcinol (2-Bromo-3:5-dihydroxytoluene). (cf. Chakravarti and Mukerjee, J. Indian Chem. Soc., 1937, 14, 725). - A stream of carbon dioxide was passed through bromine (0.45 g.) into a solution of orcinol (1 g.) in water (20 c.c.). When the bromine was completely absorbed, the solution was concentrated to small bulk under reduced pressure and extracted with chloroform (3 x 15 c.c.). The combined extract was washed with water, dried (Na_2SO_4) and evaporated under reduced pressure to give a gummy solid (0.317 g.). Recrystallisation from benzene-light petroleum (b.p. 60-80°) gave bromoorcinol as needles, m.p. 139-142° (lit., m.p. 142°).

2-Bromo-3:5-dimethoxytoluene. - 3-Bromo-4:6-dimethoxy-2-methylbenzoic acid (200 mg.) was heated with quinoline (5 c.c.) and copper powder (100 mg.) at 210-230° (bath temperature) for 1 hr. The cooled mixture was diluted with ether (20 c.c.) and the ethereal solution successively washed with 3N-hydrochloric acid, 10% aqueous sodium hydroxide and water, and dried (Na_2SO_4). Removal of the ether gave a light-brown oil which solidified. Crystallisation from aqueous ethanol followed by sublimation at $70°/10^{-3}$ mm. gave 2-bromo-3:5-dimethoxytoluene (60 mg.). The m.p. 53.5-54.5° was undepressed on admixture with a synthetic specimen, m.p. 51° (lit., m.p. 57°), prepared[19] by methylation of bromoorcinol.

5:7-Dimethoxyphthalide. - A suspension of 4-bromo-5:7--dimethoxyphthalide (1.56 g.) in dry ethyl acetate (250 c.c.) was shaken with hydrogen at room temperature and pressure in the presence of palladised charcoal (1.2g; 2.5% of palladium chloride on charcoal) and magnesium oxide (3.0 g.). When absorption of hydrogen was complete (ca. 12 hr.) the mixture was filtered (filtrate B) and the insoluble material extracted with boiling chloroform (3 x 100 c.c.). The combined extracts together with filtrate B were evaporated under reduced pressure to give a solid which was crystallised from chloroform-methanol to give 5:7-dimethoxyphthalide (1.02 g.) as stout needles, m.p. 151-153° (Found: C,61.5; H,5.25. $C_{10}H_{10}O_4$ requires C,61.85; H,5.2%), light absorption: Max. at 2165 (ε = 37,600), 2560 (ε = 15,900) and 2900 Å. (ε = 5,300).

3:5-Dimethoxyphthalic Acid. - A solution of 5:7-dimethoxyphthalide (50 mg.) in hot aqueous sodium hydroxide (10 c.c.; 2N) was treated dropwise with stirring with aqueous potassium permanganate (4.6 c.c.; 5%). The mixture was heated on the steam-bath for 10 min., cooled and filtered from manganese dioxide. The residue was washed with water and the combined filtrate and washings were evaporated to small bulk under reduced pressure. Acidification (Congo red) with hydrochloric acid (d,1.15) gave 3:5-dimethoxyphthalic acid (50 mg.) as needles, m.p. 159° (decomp.) alone or mixed with an authentic sample [lit.,[21] m.p. 158° (decomp.)].

4-Chloromethyl-5:7-dimethoxyphthalide. - The following modification of the method of Wilson et al., (J. Org. Chem., 1951, 16, 792) for the preparation of meconin proved to be the most successful chloromethylation technique. 5:7-Dimethoxyphthalide (100 mg.) was suspended in hydrochloric acid (0.2 c.c.; d,1.15) and aqueous formaldehyde (0.1 c.c.; 40%), and the mixture treated with dry hydrogen chloride at 0° for 35 min. After being kept overnight at room temperature, the solid was triturated with water and immediately extracted with chloroform. The extract was washed with water, dried (Na_2SO_4) and evaporated to give a white solid. Crystallisation of the solid from ethyl acetate-light petroleum (b.p. 60-80°) gave 4-chloromethyl-5:7-dimethoxyphthalide (110 mg.) as prisms, m.p. 187.5-189° (Found: C,54.4; H,4.7. $C_{11}H_{11}O_4Cl$ requires C,54.45; H,4.6%), light absorption: Max. at 2240 (ε = 38,000), 2590 (ε = 14,400) and 2950 Å. (ε = 5,000).

4-Hydroxymethyl-5:7-dimethoxyphthalide. - 4-Chloromethyl-5:7-dimethoxyphthalide (147 mg.) was refluxed for 1½ hr. with a solution of sodium carbonate (480 mg.) in water (10 c.c.). The cooled solution was extracted with chloroform (3 x 10 c.c.) and the combined extract washed with aqueous sodium hydrogen carbonate (2 x 15 c.c.; 10%), water and dried (Na_2SO_4). Evaporation of the solvent under reduced pressure gave a solid which crystallised from ethanol to give 4-hydroxymethyl-5:7-dimethoxyphthalide (48 mg.)as needles, m.p. 233.5-240°

(Found: C,59·15; H,5·4. $C_{11}H_{12}O_5$ requires C,58·9; H,5·4%), light absorption: Max. at 2210 (ε = 35,000), 2580 (ε = 13,800) and 2930 Å. (ε = 6,150).

5:7-Dimethoxyphthalan-4-carboxylic Acid. - (a) The alkaline washings from the foregoing experiment were acidified (Congo red) with hydrochloric acid (d,1·15) and extracted with chloroform (2 x 30 c.c.). The combined extract was washed with water, dried (Na_2SO_4) and the chloroform removed under reduced pressure to give a solid residue. Crystallisation from ethyl acetate gave 5:7-dimethoxyphthalan-4-carboxylic acid (51 mg.) as short needles, m.p. 221-222·5° (Found: C,59·2; H,5·6. $C_{11}H_{12}O_5$ requires C,58·9; H,5·4%), light absorption: Max. at 2160 (ε = 26,000), 2550 (ε = 10,400) and 2945 Å. (ε = 4,400).

(b) 4-Hydroxymethyl-5:7-dimethoxyphthalide (110 mg.) was heated under reflux with methanolic sodium methoxide [from sodium (50 mg.) and dry methanol (10 c.c.)] for 7½ hr. The solution was diluted with water, made acid to Congo red with hydrochloric acid (d,1·15) and extracted with chloroform (3 x 20 c.c.). The combined extract was washed with aqueous sodium hydrogen carbonate (2 x 30 c.c.) and the alkaline washings were acidified (Congo red) with hydrochloric acid (d,1·15). The acid solution was extracted with chloroform (3 x 20 c.c.) and the combined extract was washed with water, dried (Na_2SO_4) and evaporated to dryness under reduced pressure.

Crystallisation of the solid residue from ethyl acetate gave the acid as needles, m.p. 221-222° alone or mixed with the preparation from (a).

5:7-Dimethoxy-4-methylphthalide. - 4-Chloromethyl-5:7--dimethoxyphthalide (200 mg.) was shaken in dry ethyl acetate (50 c.c.) with hydrogen at room temperature and pressure in the presence of palladised charcoal (174 mg.; 2.5% of palladium chloride on charcoal) and magnesium oxide (400 mg.). Uptake of hydrogen was complete in 4½ hr. The reaction mixture was filtered and the residue well washed with boiling ethyl acetate; the combined filtrate and washings were evaporated under reduced pressure and the residue, crystallised from ethyl acetate-light petroleum (b.p. 60-80°), gave 5:7-dimethoxy-4-methylphthalide (160 mg.) as prismatic needles, m.p. 202-203° (Found: C,63.1; H,5.6. $C_{11}H_{12}O_4$ requires C,63.45; H,5.8%), light absorption: Max. at 2220 (ε = 31,000), 2600 (ε = 14,000) and 2970 Å. (ε = 7,200).

6-Chloromethyl-5:7-dimethoxy-4-methylphthalide. - 5:7-Dimethoxy-4-methylphthalide (200 mg.) was refluxed for 1 hr. with aqueous formaldehyde (2.06 c.c.; 40%) and hydrochloric acid (3.44 c.c.; d,1.15), dissolution of the starting material being accompanied by the separation of a brown oil. The mixture was cooled, diluted with water (30 c.c.), and extracted with chloroform (3 x 50 c.c.); the combined extracts were

washed with aqueous sodium hydrogen carbonate (2 x 30 c.c.; 10%), water and dried (Na_2SO_4). Removal of the chloroform under reduced pressure gave a yellow gum which was dissolved in the minimum amount of hot benzene. On the addition of light petroleum (b.p. 60-80°), 6-chloromethyl-5:7-dimethoxy-4-methylphthalide (150 mg.) gradually crystallised as needles. Recrystallisation from light petroleum (b.p. 60-80°) alone gave needles, m.p. 107-107.5; the compound sublimed at 100°/10^{-3} mm. (Found: C,56.8; H,5.35. $C_{12}H_{13}O_4Cl$ requires C,56.15; H,5.1%), light absorption: Max. at 2210 (ε = 40,000), 2450 (ε = 8,600) and 2960 Å (ε= 2,400).

6-Cyanomethyl-5:7-dimethoxy-4-methylphthalide. - A solution of 6-chloromethyl-5:7-dimethoxy-4-methylphthalide (200 mg.) in ethanol (10 c.c.) was added during ½ hr. to a cold solution of potassium cyanide (65 mg.) in water (1 c.c.) and the mixture heated on the steam-bath for 4 hr., during which a gradual precipitation of salt took place. The cooled mixture was filtered and the residue washed with ethanol. The combined filtrate and washings were acidified (Congo red) with hydrochloric acid (d,1.15), the filtered solution was concentrated under reduced pressure to ca. 5 c.c., and the concentrate diluted with water until precipitation commenced. The precipitate was collected, washed with aqueous ethanol and crystallised from benzene-light petroleum (b.p.60-80°) to give

6-cyanomethyl-5:7-dimethoxy-4-methylphthalide (96 mg.) as needles, m.p. 129-131.5° (Found: C,63.2; H,5.3. $C_{13}H_{13}O_6N$ requires C,63.15; H,5.3%), light absorption: Max. at 2140 (ε = 41,000), 2460 (ε = 10,000) and 2915 Å. (ε = 3,150). The infrared spectrum of the compound in carbon tetrachloride solution shows a weak band at 2222 $cm.^{-1}$ characteristic of the C≡N stretching vibration.

6-Carboxymethyl-5:7-dimethoxy-4-methylphthalide. - 6-Cyanomethyl-5:7-dimethoxy-4-methylphthalide (60 mg.) was heated under reflux for 2 hr. with aqueous potassium hydroxide (10 c.c.; 10%). The cooled solution was acidified (Congo red) with hydrochloric acid (d,1.15) and the precipitate extracted with chloroform (2 x 20 c.c.). The combined extract was washed with aqueous sodium hydrogen carbonate (2 x 20 c.c.; 10%) and the alkaline washings were acidified (Congo red) with hydrochloric acid. Extraction of the acid solution with chloroform (2 x 30 c.c.) followed by evaporation of the dried (Na_2SO_4) extract under reduced pressure gave 6-carboxymethyl--5:7-dimethoxy-4-methylphthalide (47 mg.) which separated from benzene-light petroleum (b.p. 60-80°) as needles, m.p. 152-153° (Found: C,58.6; H,5.5. $C_{13}H_{14}O_8$ requires C,58.6; H,5.3%), light absorption: Max. at 2160 (ε = 43,000), 2500 (ε = 11,000) and 2930 Å. (ε = 3,300). The acid on being mixed with 6-carboxymethyl-5:7-dimethoxy-4-methylphthalide, m.p. 152-153° (lit.,[6] m.p. 153°), obtained by ozonolysis of mycophenolic acid

methyl ether according to the method of Raistrick et. al.[6], had m.p. 152-153° and the infrared spectra of both acids determined in nujol were identical. The methyl esters, prepared with diazomethane and crystallised from methanol, had m.p. 92-93.5° (synthetic) and 93-94° (from degradation; lit.,[6] m.p. 94-95°) and the m.p.'s were undepressed on mixing. The m.p. of the synthetic acid was not depressed on mixing with 6-carboxymethyl-5:7-dimethoxy-4-methylphthalide prepared[6] by alkaline iodine oxidation of 6-formylmethyl-5:7-dimethoxy-4-methylphthalide.

6-Hydroxymethyl-5:7-dimethoxy-4-methylphthalide. - 6-Chloromethyl-5:7-dimethoxy-4-methylphthalide (69 mg.) was refluxed with aqueous sodium carbonate (10 c.c.; 10%) for 1 hr. The cooled solution was filtered, acidified (Congo red) with hydrochloric acid (d,1.15) and extracted with chloroform (3 x 10 c.c.). The combined extract was washed with water, dried (Na_2SO_4) and evaporated to dryness under reduced pressure. The resultant gum solidified on standing and recrystallisation of the solid from benzene-light petroleum (b.p. 60-80°) gave 6-hydroxymethyl-5:7-dimethoxy-4-methylphthalide as needles, m.p. 103-104° (Found: C,60.5; H,6.3. $C_{12}H_{14}O_5$ requires C,60.5; H,5.9%), light absorption: Max. at 2150 (ε = 31,000), 2490 (ε = 7,500) and 2935 Å. (ε = 2,500).

6-Formyl-5:7-dimethoxy-4-methylphthalide. - A solution of crude 6-hydroxymethyl-5:7-dimethoxy-4-methylphthalide

(420 mg.) in glacial acetic acid (20 c.c.) was treated, at room temperature with stirring, during 3 min. with a solution of chromium trioxide (500 mg.) in acetic acid (10 c.c.). Five minutes after the addition had been completed, the solution was diluted with water (20 c.c.) and extracted with chloroform (3 x 30 c.c.). The combined extract was washed with aqueous sodium hydrogen carbonate (2 x 80 c.c.; 10%) and water, and dried (Na_2SO_4). Removal of the chloroform under reduced pressure gave a yellow gum which rapidly solidified. The solid was extracted with boiling light petroleum (b.p. 80-100°). On standing, the extract deposited needles which were thrice recrystallised from the same solvent and then sublimed at $100°/10^{-3}$ mm. to give 6-formyl-5:7-dimethoxy-4-methylphthalide (60 mg.), m.p. 131.5-133° (Found: C,60.9; H,5.2. $C_{12}H_{12}O_5$ requires C,61.0; H,5.1%), light absorption: Max. at 2320 (ε = 28,000) and 3100 (ε = 2,700) and inflexion at 2040-2100 Å. (ε = 13,000). The 2:4-dinitrophenylhydrazone, which crystallised from benzene as orange needles, had m.p. 216° (decomp.) with previous softening at 213°. Using the procedure of Haistrick et al.,[6] an attempt was made at the preparation of 6-formyl-5:7-dimethoxy-4-methylphthalide by side-chain degradation of mycophenolic acid methyl ether, but the product could not be isolated in a crystalline form.

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PART II.

STUDIES ON ALKYL-OXYGEN BOND FISSION IN PHTHALIDES.

SUMMARY

1. The constitution of gladiolic acid, a mould metabolite of *P. gladioli* Machacek, is discussed and a new synthesis of the acid from 5-methoxy-6-methylphthalan-4-carboxylic acid is described.

2. A novel preparation of 7-ethoxy-6-methoxyphthalide is described.

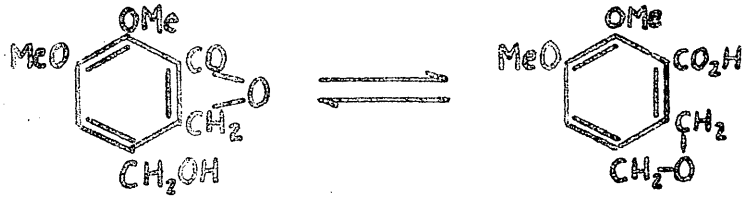
3. The preparation of dimethyl and diethyl α-(1:3-dihydro-6:7-dimethoxy-1-oxo-4-benzo[c]furylmethyl)malonate from 4-chloromethylmeconin is described together with isomerisation of the esters to the corresponding indane-4-carboxylic acids on treatment with alcoholic sodium alkoxide. The mechanism of this isomerisation and of the analogous hydroxymethylphthalide-carboxyphthalan isomerisation is discussed.

4. The preparation of the parent phthalan-4-carboxylic acid is described and the attempted isomerisation of this acid to 4-hydroxymethylphthalide is reported.

INTRODUCTION

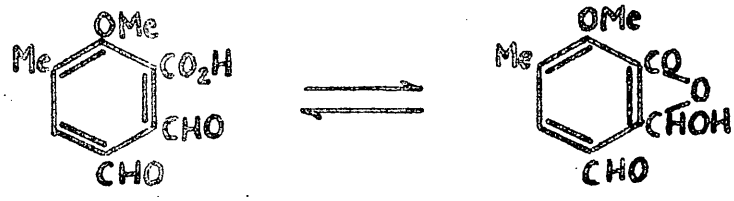
The Hydroxymethylphthalide - Carboxyphthalan Isomerisation.

The isomerisation of 6:7-dimethoxy-4-hydroxymethylphthalide (I) to 5:6-dimethoxyphthalan-4-carboxylic acid (II) was discovered by Brown and Newbold[1] in the course of model experiments on a synthetical route to gladiolic acid.



(I) (II)

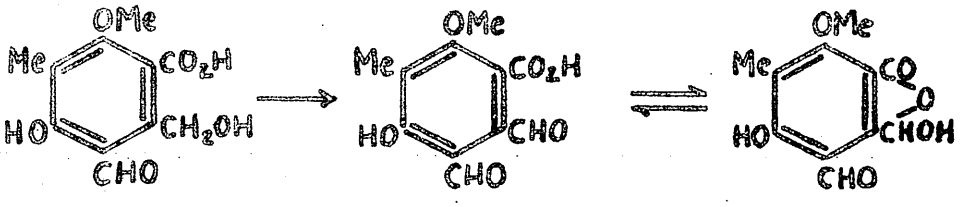
Gladiolic acid (III) is a metabolite of strains of Penicillium gladioli and it possesses antifungal and antibacterial properties. The structure of the acid, first isolated by Brian et al.[2], was independently elucidated by Raistrick and Ross[3] and Grove[4] and finally confirmed by synthesis by Brown and Newbold.[5]



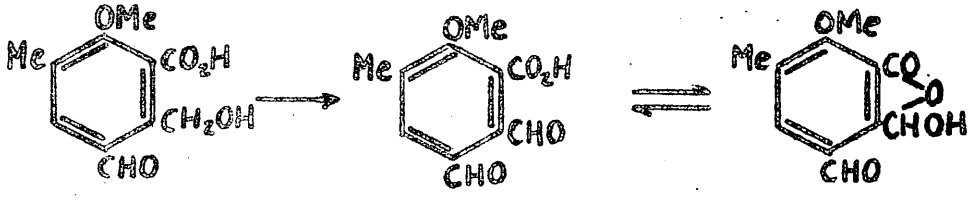
(III)

Raistrick and Ross derived structure (III) from that of dihydrogladiolic (IV), a metabolite produced by P. gladioli

on altering the proportion of the constituents of the culture medium. Birkinshaw, Raistrick, Ross and Stickings[6] had shown that oxidation of cyclopolic acid (V) with potassium periodate in dilute sulphuric acid gave cyclopaldic acid (VI) and since dihydrogladiolic acid gave gladiolic acid under similar conditions, the former authors deduced the structure of gladiolic acid by analogy.



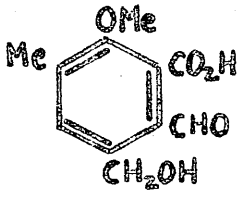
(V) (VI)



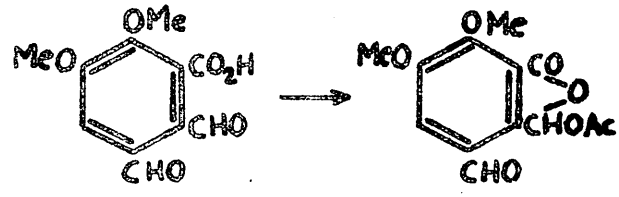
(IV) (III)

Brown and Newbold[7], however, considered structure (IV) for dihydrogladiolic acid unlikely due to the known ease of lactonization of ortho-hydroxymethylbenzoate salts on acidification. The only other alternative was structure (VII). Starting from 3-formylopianic acid (VIII), the latter authors synthesised 3-hydroxymethylopianic acid (IX) and from the similarity of its properties to those of dihydrogladiolic acid

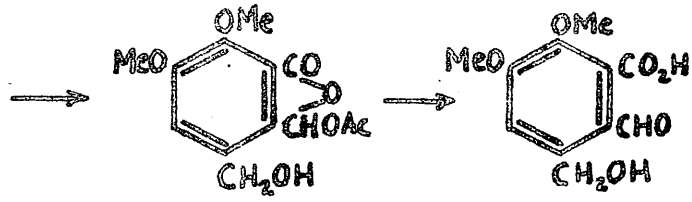
deduced the more probable structure for dihydrogladiolic acid to be (VII). Duncanson, Grove and Zealley[8] confirmed



(VII)

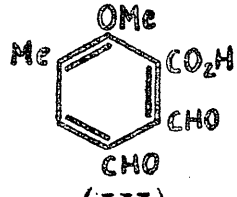


(VIII)

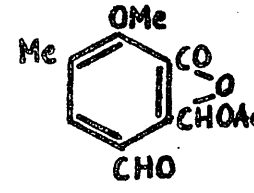


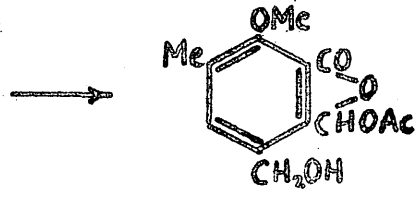
(IX)

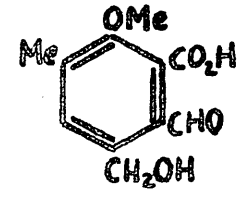
structure (VII) for dihydrogladiolic acid by synthesis from gladiolic acid (III).



(III)

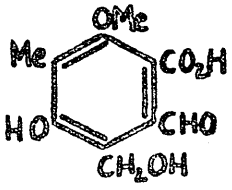






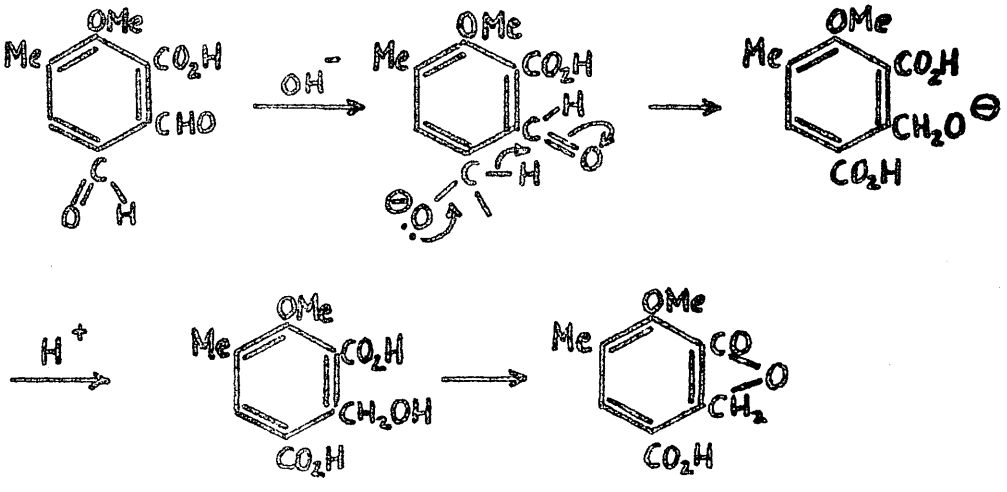
(VII)

By analogy, it has been suggested[9] that the more probable structure for the closely related metabolite cyclopolic acid[6] is (X).



(X)

Grove[4] established the structure of gladiolic acid by a study of the reactions and properties of the acid itself and of isogladiolic acid (XI), the alkaline isomerisation product of gladiolic acid. This isomerisation was explained by analogy with the mechanism advanced by Alexander[10] and Doering et al.[11], for the rearrangement of phenylglyoxal to mandelic acid. The possibility of (XII) lactonising in the



(XII) (XI)

other direction existed but Brown and Newbold[12] confirmed structure (XI) for isogladiolic acid by synthesis from 7-methoxy-6-methylphthalide (XIII). The position of the

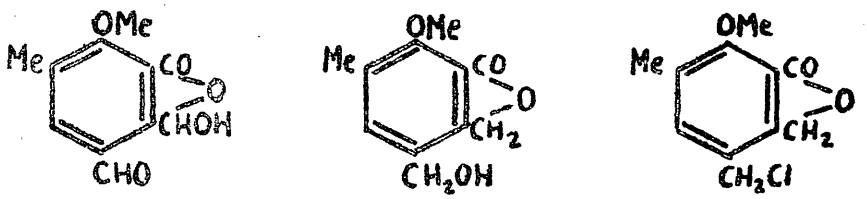
(XIII) (XIV)

(XV) (XI)

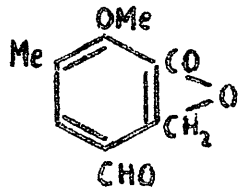
entrant chlormethyl group in (XIV) was inferred by analogy with the chloromethylation of meconin,[1] and hydrolysis of (XIV) to 4-hydroxymethyl-7-methoxy-6-methylphthalide (XV) with sodium carbonate was followed by acid potassium permanganate oxidation to isogladiolic acid (XI).

The structure for gladiolic acid thus assigned by Raistrick and Ross and by Grove was confirmed by the unambiguous synthesis of the acid by Brown and Newbold[5]. Since Raistrick and Ross had shown that oxidation of dihydrogladiolic acid with periodate gave gladiolic acid, this treatment was applied to deoxygladiolic acid (XVI) which was formed from gladiolic acid (III) by reduction with iron powder in acetic acid[4] and

by the action of N-bromosuccinimide[7] on 4-hydroxymethyl-7--methoxy-6-methylphthalide (XVII) or the 4-chloromethyl compound (XVIII), followed by hydrolysis. Prolonged oxidation with



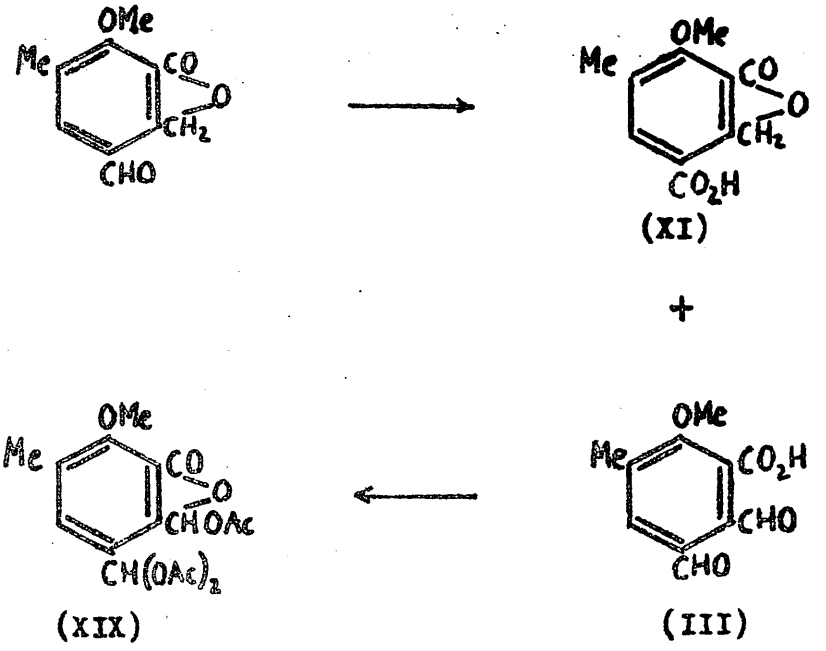
(III) (XVII) (XVIII)



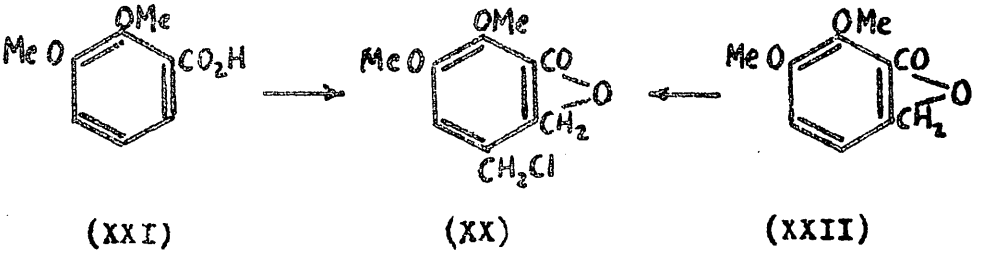
(XVI)

sodium metaperiodate in dilute sulphuric acid resulted in the partial conversion of deoxygladiolic acid into gladiolic acid (III) and isogladiolic acid (XI), the gladiolic acid being isolated through its hydrate triacetate (XIX).

Initial support for the structure of gladiolic acid was obtained by Brown and Newbold[1] from the observation of the close similarity in properties between gladiolic acid and the analogous 3-formylopianic acid (VIII). The latter compound was synthesised from 4-chloromethylmeconin (XX) which was obtained by the method of Manske and Ledingham[13] from



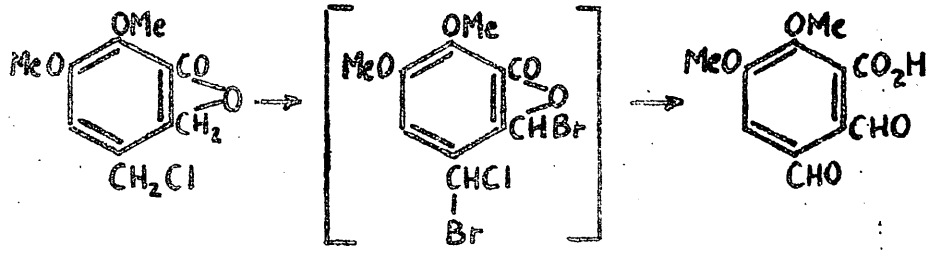
o-veratric acid (XXI) or by chloromethylation of meconin (XXII) itself. The position of the chloromethyl group in (XVII) was proved[13] by its reduction to 4-methylmeconin which



(XXI) (XX) (XXII)

was synthesised by an unambiguous route. Using the technique which was known[14] to give o-phthalaldehydic acids from phthalides, 4-chloromethylmeconin was treated with 3 mols.

of N-bromosuccinimide and the intermediate (XXIII) hydrolysed with water to 3-formylopianic acid (VIII).



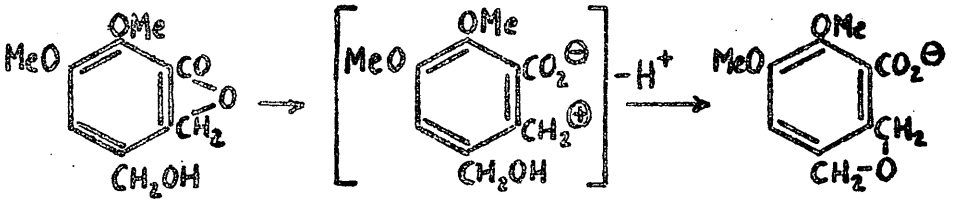
(XXIII) (VIII)

An alternative route to 3-formylopianic acid (VIII) appeared to be the direct oxidation of 4-hydroxymethylmeconin (I), readily obtained by hydrolysis of the chloromethyl compound (XX). Oppenauer oxidation[1] of (I) with benzophenone as hydrogen acceptor did not give the expected o-carboxyphthalaldehyde system but an isomeric acid. This acid was formulated as 5:6-dimethoxyphthalan-4-carboxylic acid (1:3-dihydro-5:6-dimethoxybenzo[c]furan-4-carboxylic acid) (II) and the isomerisation in the reverse direction could be effected by mineral acid. Subsequent examination showed that the isomeric acid could be produced by the action of aluminium t-butoxide in benzene alone.

Blair and Newbold[15] made a more detailed study of this novel isomerisation and noted the following variation in the percentage of 4-hydroxymethylmeconin isomerised with the reagent used.

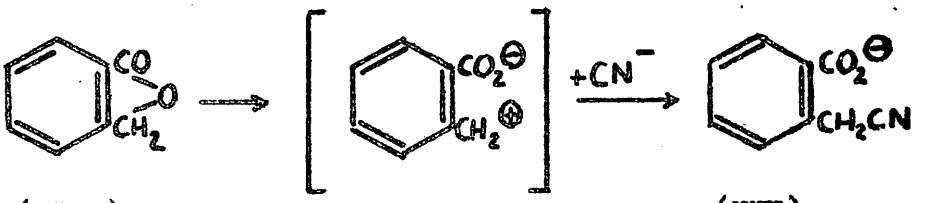
Reagent	% isomerised
NaOH aqu.	8
Na_2CO_3 aqu.	31
NaOMe in C_6H_6	39
Al t-butoxide in C_6H_6	60
NaOMe in MeOH	100

When sodium methoxide was used as the isomerising agent the absence of any ester in the product suggested that the lactone ring in (I) had undergone B_{AL} fission[18] with the formation of an intermediate (XXIV) containing a carbonium ion which reacted with the 4-hydroxymethyl group with loss of a proton. The formation of o-carboxyphenylacetonitrile (XXV) on treating phthalide (XXVI) with potassium cyanide[17] appeared



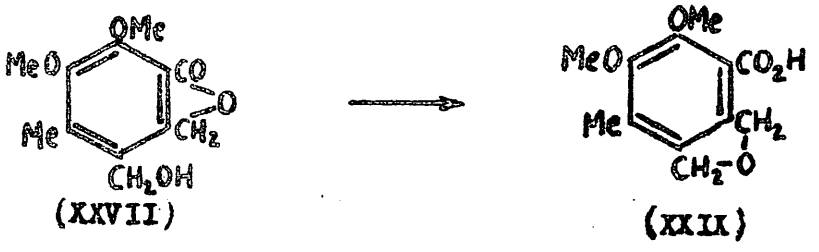
(I) (XXIV) (II)

to be a precedent for the postulated B_{AL} fission of the lactone ring.

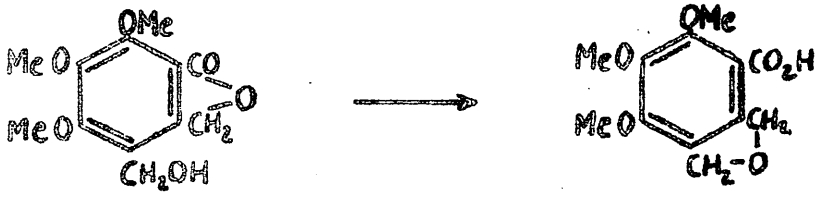


(XXVI) (XXV)

The hydroxymethylphthalide-carboxyphthalan isomerisation has been extended[18] to two more derivatives of 4-hydroxymethyl-phthalide, namely 5-methyl-6:7-dimethoxy-4-hydroxymethyl-phthalide (XXVII) and the 5:6:7-trimethoxy compound (XXVIII), partial isomerisation to the corresponding phthalan acids (XXIX) and (XXX) being obtained on refluxing with aqueous sodium carbonate

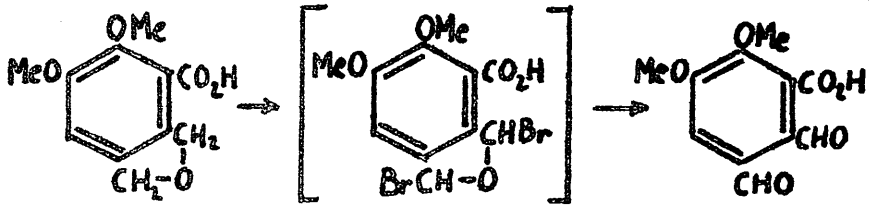


(XXVII) (XXIX)



(XXVIII) (XXX)

Although Oppenauer oxidation of 4-hydroxymethylmeconin (I) did not give the required 3-formylopianic acid (VIII), the latter compound could be obtained[1] on treatment of 5:6-dimethoxyphthalan-4-carboxylic acid (II) with 2 mols. of N-bromosuccinimide followed by hydrolysis of the intermediate (XXXI).

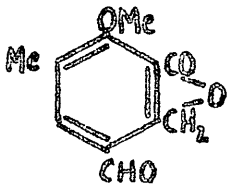

OMe
MeO
CO2H
CH2
CH2-O
OMe
MeO
CO2H
CHBr
BrCH-O
OMe
MeO
CO2H
CHO
CHO

(II) (XXXI) (VIII)

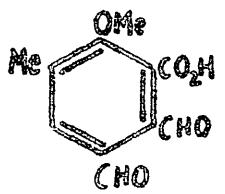
THEORETICAL

Synthesis of Gladiolic Acid.

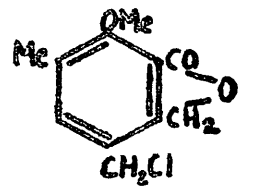
Although gladiolic acid (III) has been synthesised[5] by periodate oxidation of 4-formyl-7-methoxy-6-methylphthalide (XVI) initial attempts at this synthesis were directed[7] towards the N-bromosuccinimide treatment of the 4-chloromethyl compound (XVIII). Such treatment, followed by hydrolysis, had been effective[1] for the preparation of 3-formylopianic (VIII) from 4-chloromethylmeconin (XX) but failed to produce gladiolic acid. A similar lack of success had been noted[18] in the attempted preparation of 3-formyl-4-methylopianic acid and its 4-methoxy analogue from the corresponding 4-chloromethyl compounds.



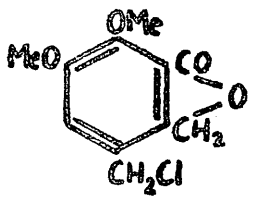
(XVI)



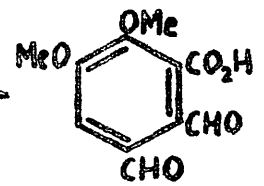
(III)



(XVIII)



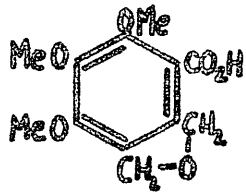
(XX)



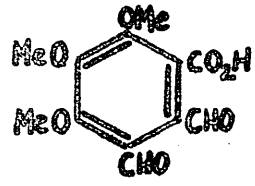
(VIII)

Another method for the preparation of 6-carboxyphthal-aldehydes was the treatment of phthalan-4-carboxylic acid

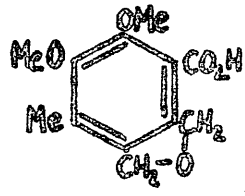
derivatives with N-bromosuccinimide followed by hydrolysis of the intermediate. First used for the preparation of 3-formylopianic acid,[1] this method was subsequently employed by Blair and Newbold[15] for the preparation of 3-formyl-4--methoxyopianic acid (XXXII) and its 4-methyl analogue (XXXIII) from the corresponding phthalan acids (XXXIV) and (XXXV).



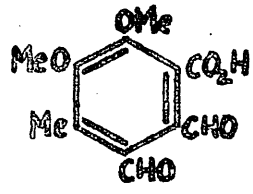
(XXXIV)



(XXXII)

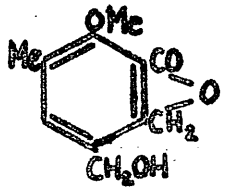


(XXXV)

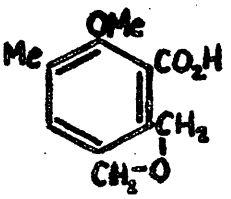


(XXXIII)

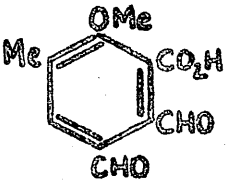
This preparative route has now been successfully applied to the synthesis of gladiolic acid. The 5-methoxy-6-methylphthalan-4-carboxylic acid (XXXVI) required was readily obtained by isomerising the known 4-hydroxymethyl-7-methoxy-6--methylphthalide[12] (XVII) with sodium methoxide. This isomerisation was yet another example of the hydroxymethylphthalide-phthalancarboxylic acid rearrangement[1, 15].



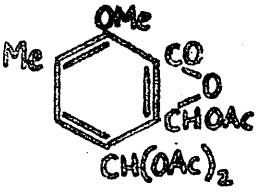
(XVII)



(XXXVI)



(III)



(XIX)

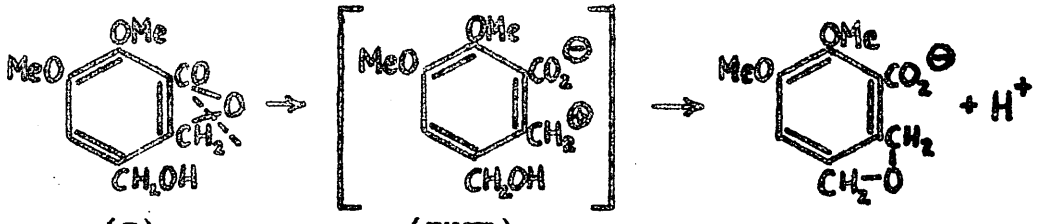
Treatment of the carboxyphthalan (XXXVI) with N-bromo-succinimide and subsequent hydrolysis of the intermediate gave gladiolic acid (III) which was isolated as the hydrate triacetate (3-acetoxy-4-diacetoxymethyl-7-methoxy-6-methyl-phthalide) (XIX). The triacetate has been hydrolysed to gladiolic acid by hot mineral acid[5]. The yield of gladiolic acid hydrate triacetate by this method was 11% as compared with 10% by the less convenient periodate oxidation method.[5]

Synthesis of Indane Derivatives.

The isomerisation of a 4-hydroxymethylphthalide to a phthalan-4-carboxylic acid was first obtained for the 6:7-dimethoxy derivative of the former by Brown and Newbold[1] using aluminium t-butoxide in benzene. The scope of this isomerisation has now been widened to include the 5:6:7-

-trimethoxy-[15], 5-methyl-6:7-dimethoxy-[15], 6-methyl-7-methoxy-(p.45), and 5:7-dimethoxy- (p.12) derivatives of 4-hydroxymethylphthalide.

Blair and Newbold[15] studied the behaviour of 4-hydroxymethylmesconin (I) with different bases and found that sodium methoxide in methanol was a more efficient isomerising agent than aluminium t-butoxide, the yield of phthalan in the former case being quantitative. Noting that there was no ester formation with sodium methoxide as would be expected for B_{AC}[16] fission of the lactone ring, these authors suggested that the isomerisation proceeded by B_{AL} fission of the phthalide ring with the production of a carbonium ion (XXIV) which then formed the phthalan ring by interaction with the 4-hydroxymethyl group.



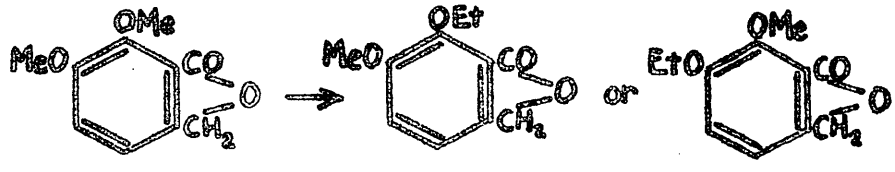
(I) (XXIV)

A reaction which may also involve B_{AL} fission of the phthalide ring was the preparation[17] of o-carboxyphenylacetonitrile (XXV) from phthalide.

Working on this hypothesis of carbonium ion-formation, it seemed of interest to attempt to unite the carbonium ion with a suitable carbanion such as that derived from a malonic ester.

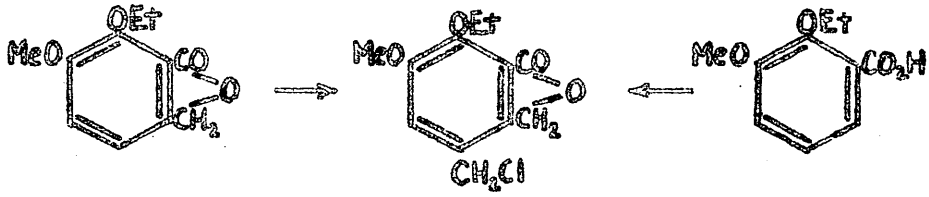
(XXV)

When 4-hydroxymethylmeconin (I) was heated with an excess of methyl sodiomalonate in methanol, however, there was no evidence of any carbonium ion-carbanion union, only the normal rearrangment product (II) being isolated. When meconin itself was reacted with ethyl sodiomalonate, a lactone m.p. 66-67.5° having the molecular formula $C_{11}H_{12}O_4$ was produced in good yield. Subsequent examination showed that the same lactone was produced when meconin was treated with ethyl sodioacetoacetate or ethanolic sodium ethoxide. The analytical data indicated that substitution of an ethoxyl group for a methoxyl group had taken place and thus the lactone $C_{11}H_{12}O_4$ was 7-ethoxy-6-methoxyphthalide (XXXVII) or the 7-methoxy-6-ethoxy isomer (XXXVIII).



(XXXVII) (XXXVIII)

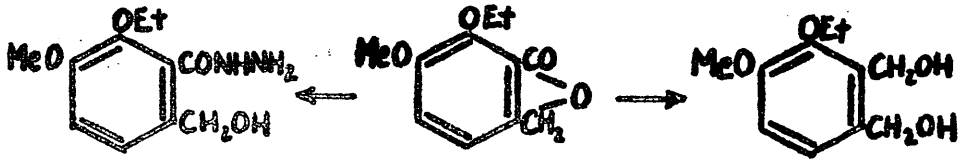
The preparation of (XXXVII), m.p. 68-69°, by ethylation of 7-normeconin[18,19] has been reported[18] but its structure has not been rigidly established. Examination of the infrared absorption spectra of a specimen of 7-normeconin in dilute solution showed strong phthalide carbonyl stretching frequencies at 1751(CCl_4) and 1739($CHCl_3$) cm.$^{-1}$, confirming the presence of the o-hydroxycarbonyl system assigned.[20] Thus the structure of the ether (XXXVII) was rigidly established (cf. refs. 18 and 21). Confirmation of the assignment of structure (XXXVII) to the lactone m.p. 66-67.5° was given by the identity of its chloromethyl derivative with a sample of 4-chloromethyl-6-methoxy--7-ethoxyphthalide (XXXIX) prepared[18] from 2-ethoxy-3-methoxybenzoic acid (XL). The phthalide (XXXVII) was subsequently



(XXXVII) (XXXIX) (XL)

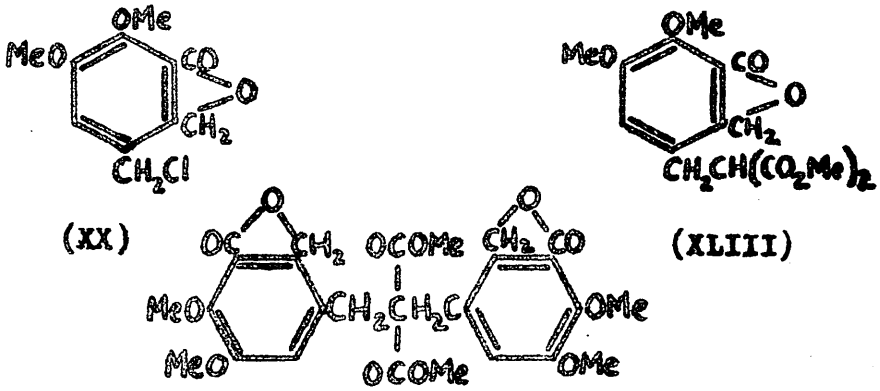
characterised by conversion into 2-ethoxy-6-hydroxymethyl-3--methoxybenzhydrazide (XLI) and by reduction with lithium aluminium hydride to 3-ethoxy-4-methoxyphthalyl alcohol (XLII).

Attention was then turned to the possibility of reacting the carbonium ion produced by B_{AL} fission with a suitably placed carbanion from an α-substituted malonic ester in the



(XLI) (XXXVII) (XLII)

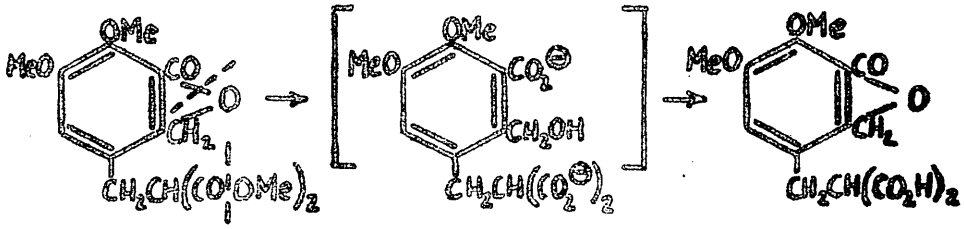
same molecule. Such a molecule was dimethyl α-(1:3-dihydro--6:7-dimethoxy-1-oxo-4-benzo[c]furylmethyl)malonate (XLIII) which was obtained as the major product of the reaction between equimolecular proportions of 4-chloromethylmeconin (XX) and methyl sodiomalonate. Reaction did not stop at the mono-α-substituted malonic ester however, a small proportion of methyl sodiomalonate reacted with (XX) to give the corresponding αα-di-(1:3-dihydro-6:7-dimethoxy-1-oxo-4-benzo[c]furylmethyl) malonate (XLIV) which was characterised by hydrolysis to the malonic acid which was esterified to the original ester on treatment with diazomethane.



(XX) (XLIII)

(XLIV)

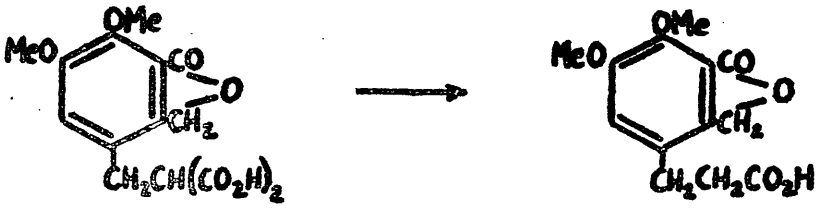
The fact that the mono-α-substituted malonic ester (XLIII) gave on hydrolysis with aqueous sodium hydroxide α-(1:3-dihydro-6:7-dimethoxy-1-oxo-4-benzo[c]furylmethyl) malonic acid (XLV) which was esterified with diazomethane to the original ester (XLIII) indicated that the carbon skeleton had suffered no change on aqueous alkaline treatment as would be expected of B_{AC} 2 fission of the phthalide ring.



(XLIII) (XLV)

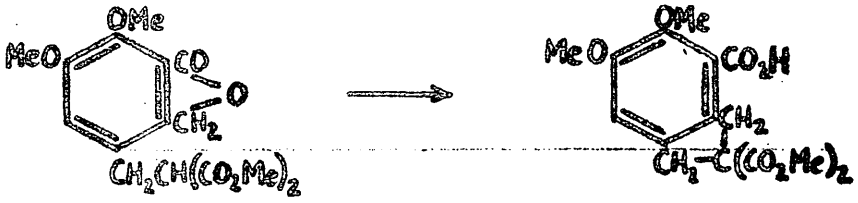
The acid (XLV) underwent decarboxylation to give β-(1:3-dihydro-6:7-dimethoxy-1-oxo-4-benzo[c]furyl)propionic acid (XLVI) which showed two infrared absorption bands in the carbonyl stretching region corresponding to the phthalide carbonyl (1754 $cm.^{-1}$) and the carboxyl (1695 $cm.^{-1}$) groups. The methyl ester, obtained by the action of diazomethane, showed absorptions corresponding to the phthalide carbonyl (1750 $cm.^{-1}$) and methoxycarbonyl (1718 $cm.^{-1}$) groups. Both determinations were effected in nujol mull.

When the substituted malonic ester (XLIII) was treated with 1 mol. of sodium methoxide in methanol, it was partially converted into an isomeric monocarboxylic acid which we



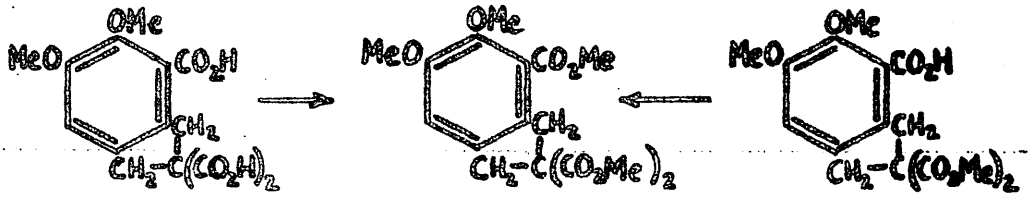
(XLV) (XLVI)

formulate as 5:6-dimethoxy-2:2-di(methoxycarbonyl)indane-4--carboxylic acid (XLVII). The latter compound was also obtained as the acidic fraction of the product from reacting 4-chloromethylmeconin with methyl sodiomalonate. Alkaline



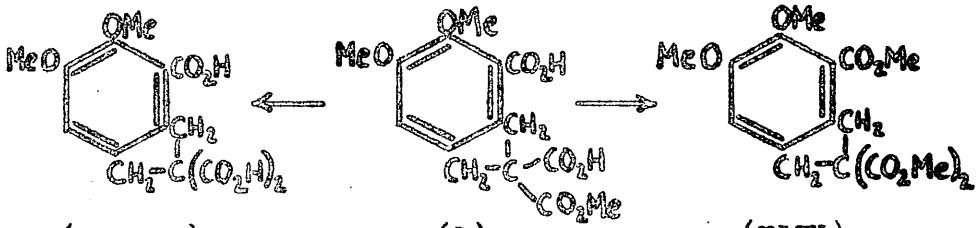
(XLIII) (XLVII)

hydrolysis of the acid (XLVII) gave 5:6-dimethoxyindane -2:2:4-tricarboxylic acid (XLVIII) which was readily esterified to the trimethyl ester (XLIX), obtained alternatively by esterifying the acid ester (XLVII).



(XLVIII) (XLIX) (XLVII)

When dimethyl α-(1:3-dihydro-6:7-dimethoxy-1-oxo-4-benzo [c]furylmethyl)malonate (XLIII) was treated with an excess of methanolic sodium methoxide an acidic product was obtained in good yield which was not the indane acid (XLVII) but a dicarboxylic acid, $C_{18}H_{16}O_8$. Since the latter was hydrolysed to 5:6-dimethoxyindane-2:2:4-tricarboxylic acid (XLVIII) and was esterified with diazomethane to the triester (XLIX) we formulate it as 5:6-dimethoxy-2-methoxycarbonylindane-2:4-dicarboxylic acid (L).



(XLVIII) (L) (XLIX)

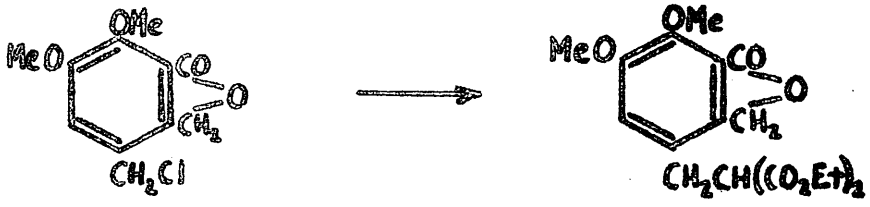
Presumably the latter acid is formed from 5:6-dimethoxy-2:2-di(methoxycarbonyl)indane-4-carboxylic acid by partial hydrolysis brought about by traces of water in the redistilled commercial alcohol used as solvent.

The tricarboxylic acid (XLVIII) readily lost 1 mol. of carbon dioxide on heating to give the expected 5:6-dimethoxyindane-2:4-dicarboxylic acid (LI) which showed an infrared absorption band at 1695 cm.$^{-1}$ (carboxylic acid) in nujol mull thus confirming the absence of the phthalide ring system.

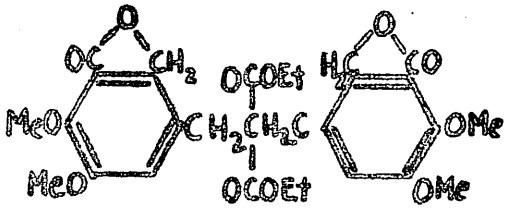
The ethyl esters (LII) and (LIII), analogous with the compounds (XLIII) and (XLIV) in the methyl ester series

(XLVIII) (LI)

were obtained by reacting 4-chloromethyl meconin with ethyl sodiomalonate.



(LII)

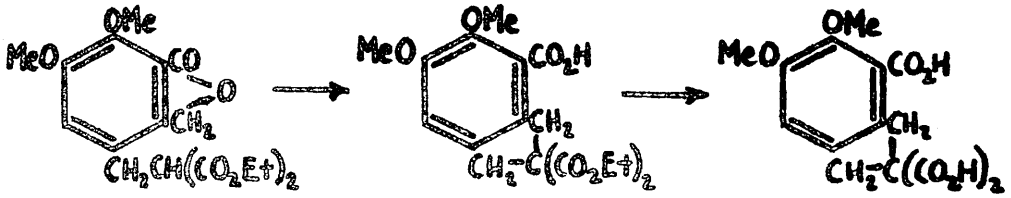


(LIII)

The possibility of ether interchange as experienced with meconin was ruled out by the fact that the mono-α-substituted malonic ester (LII) underwent hydrolysis to give α-(1:3-dihydro-6:7-dimethoxy-1-oxo-4-benzo[c]furylmethyl) malonic acid.

The ethyl ester (LII) also underwent isomerisation on treatment with sodium ethoxide in ethanol to give 5:6--dimethoxy-2:2-di(ethoxycarbonyl)indane-4-carboxylic acid

(LIV) which was hydrolysed to the tricarboxylic acid (XLVIII).



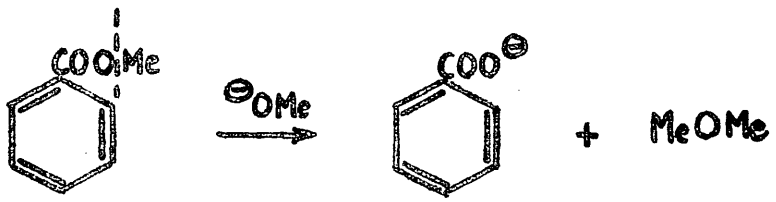
(LII) (LIV) (XLVIII)

In order to obtain further confirmation of the structures assigned to the phthalide-malonic esters and the indane isomerisation products, the ultraviolet light absorption of both series of compounds was examined. All the compounds containing the phthalide ring [(XLIII), (XLV), (XLVI) and (XLVI Me ester)]showed a band at 3110-3130 Å. which compared with that in meconin[1] at 3080 Å. whilst the compounds in the indane series [(XLVII), (XLVIII), (L) and (LI)] showed a band at 2960-2990 Å.which compared with that in o-veratric acid (2:3-dimethoxybenzoic acid) at 2950 Å. In addition, electrometric titrations were carried out on all the carboxylic acids obtained and, with the exception of that for (XLV) which was 6% high, the equivalents obtained were in close agreement with the calculated values, thus supporting the structures assigned.

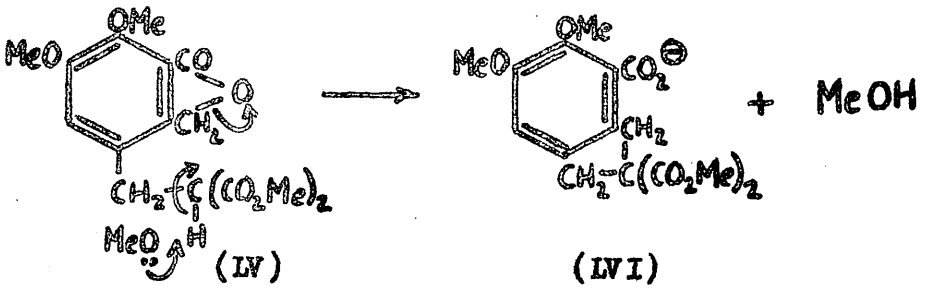
The Mechanism of Isomerisation.

Although the formation of a carbonium ion was used as a working hypothesis in explaining the isomerisation of the phthalide-malonic ester to the indane derivative, it was

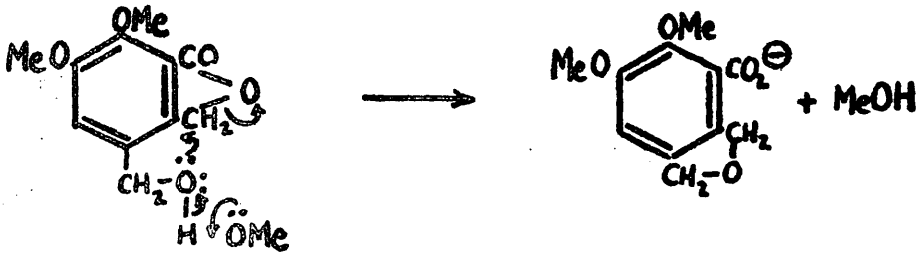
difficult to visualise B_{AL} 2 fission of the phthalide ring brought about by methoxide ion which did not result in the formation of an ether as illustrated by the hydrolysis of methyl benzoate to yield dimethyl ether.[22]



We therefore propose that the isomerisation is best explained as a concerted electron-transfer of the type shown (LV) → (LVI), the site of attack of the methoxide ion being the labile α-hydrogen atom of the malonic ester.

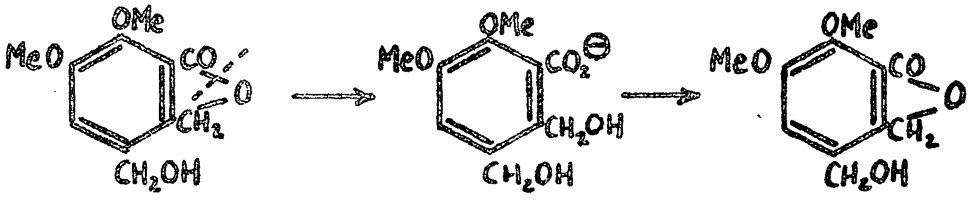


It follows that the hydroxymethylphthalide-phthalan-carboxylic acid rearrangement as brought about by the methoxide ion is now represented by (LVII) → (LVIII).



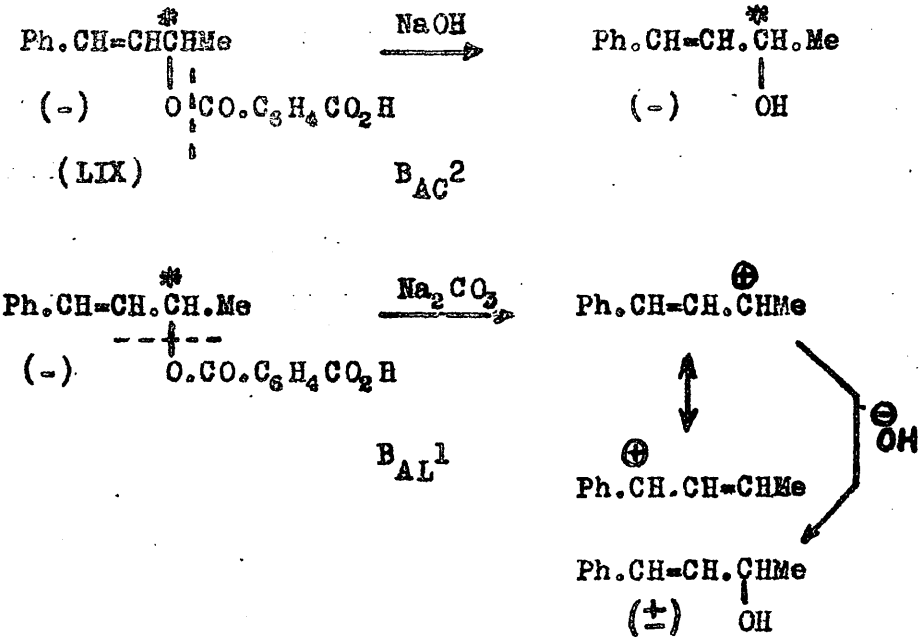
(LVII) (LVIII)

The latter isomerisation has also been effected[15] by aqueous sodium hydroxide and carbonate. The low yield (8%) of phthalan acid obtained with sodium hydroxide suggested that hydrolysis of the lactone ring was taking place almost exclusively by the normal $B_{AC}2$ fission which would entail the regeneration of the starting material on acidification.



The small amount of phthalan acid formed could not be accounted for by a mechanism involving $B_{AL}2$ fission since this again would result in the regeneration of the starting material.

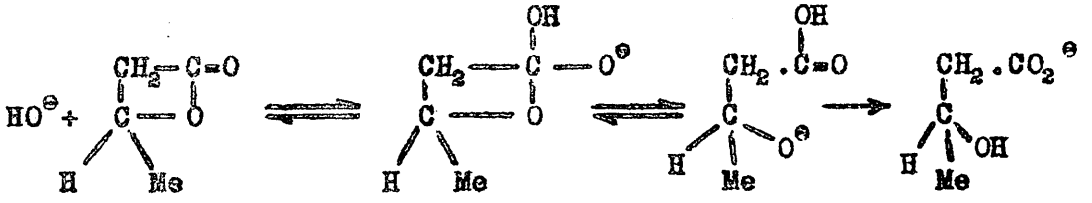
We therefore propose that the much slower $B_{AL}1$ fission takes place to a limited extent to give the intermediate carbonium ion (XXIV) which then reacts according to the scheme of Blair and Newbold[18]. With aqueous sodium carbonate, the 31% yield of phthalan acid suggests that, with weaker alkali, the rate of the second-order process is much reduced with a corresponding increase in the rate of $B_{AL}1$ fission. Support for these conclusions is given by the experiences of Kenyon *et al.*[23], in the hydrolysis of optically active methylphenylallyl esters. Hydrolysis of (-)-1-methyl-3-phenylallyl hydrogen phthalate (LIX) with 5N alcoholic sodium hydroxide gave the (-) alcohol, together with a little racemate, while hydrolysis with aqueous sodium carbonate gave the racemic alcohol.



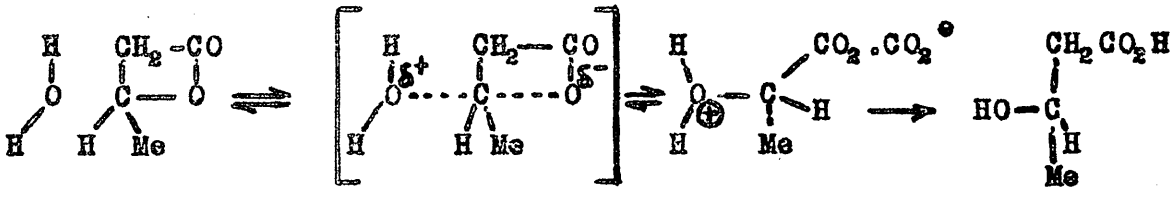
Thus the former hydrolysis proceeded almost exclusively by $B_{AC}2$ fission which could not racemise the alcohol and in

the latter hydrolysis, B_{AL} 1 fission was the controlling type which lead to the formation of a carbonium ion and hence a racemic alcohol.

The proposal of simultaneous occurrence of B_{AC} 2 fission with B_{AL} 1 fission of appreciable rate gains support from the observations of Olson and Miller[24] that while β-butyrolactone was hydrolysed with sodium hydroxide to β-hydroxybutyric acid with 98% retention of configuration of the asymmetric centre and hydrolysed with water to β-hydroxybutyric acid with 99% inversion, hydrolysis with carbonate buffer of pH9 gave 50% inversion.



B_{AC} 2

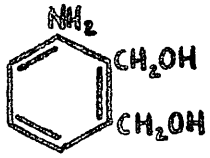


B_{AL} 2

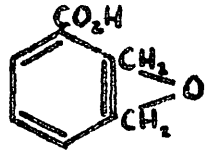
This would imply simultaneous B_{AC} 2 and B_{AL} 2 fission at equal rates.

The Preparation of Phthalan-4-carboxylic Acid.

Since the isomerisation of 4-hydroxymethyl phthalides to phthalan-4-carboxylic acids had been observed only in the case of benzene ring-substituted compounds (cf. p.46), it seemed of interest to prepare the parent phthalan-4-carboxylic acid (LX) and examine its properties, particularly its behaviour on treatment with acid. The route envisaged for this preparation required 3-aminophthalyl alcohol (LXI) as starting material.



(LXI)

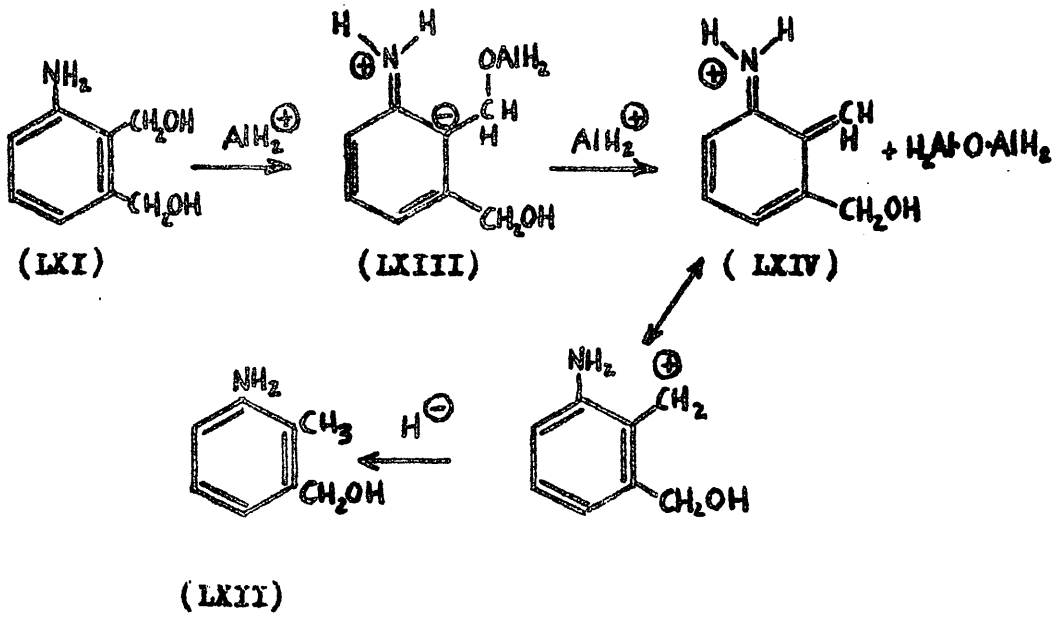


(LX)

Lithium aluminium hydride reduction of dimethyl 3-aminophthalate[28] in refluxing ethereal solution gave two products which were separated by crystallisation and chromatography. The first product was the expected 3-aminophthalyl alcohol (LXI) while the other was a product of further reduction having the formula $C_8H_{11}ON$ and m.p. 106-107°. In an attempt to reduce the yield of the undesired secondary reduction product the reaction was carried out at 0° but the yield of 3-aminophthalyl alcohol was only slightly higher. That 3-aminophthalyl alcohol was the intermediate in the preparation

of the compound $C_8H_{11}ON$ was demonstrated by the fact that more vigorous lithium aluminium hydride reduction of the former produced a partial conversion into the latter.

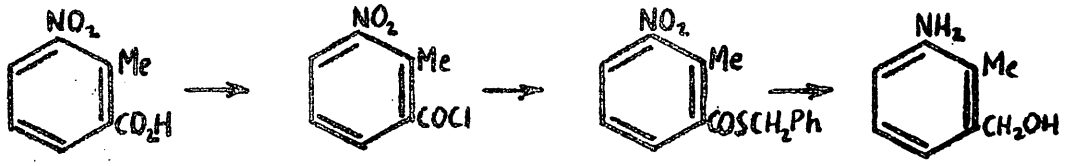
Conover and Tarbell[26] have shown that while the lithium aluminium hydride reduction of benzenoid carbonyl compounds with o- or p-amino groups gives the corresponding alcohol under normal conditions, prolonged reaction periods at elevated temperatures result in the corresponding de-oxy compound: these authors used much more drastic conditions than those described in this work. It therefore appeared that the compound $C_8H_{11}ON$ must be formulated as 3-amino-2-methylbenzyl-alcohol (LXII). Applying the hypothesis of Conover and Tarbell, the mechanism of the production of the de-oxy compound from the diol may be represented by the sequence (LXI)→(LXII) shown below.



(LXI) (LXIII) (LXIV)

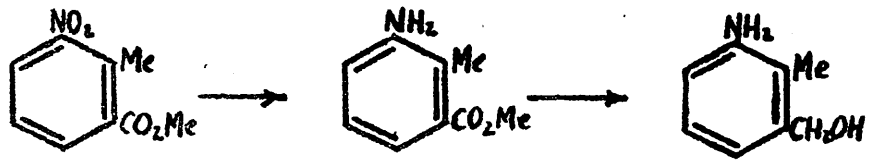
(LXII)

The electrophilic attack of the $AlH_2^{\oplus}$ ion on the diol (LXI) would be concentrated on the oxygen atom of the o-hydroxymethyl group by virtue of the enhanced electron density in the o-position. Further electrophilic attack of the $AlH_2^{\oplus}$ ion on the o-methylene group in (LXIII) followed by fission of the carbon-oxygen bond gives the resonance-stabilised carbonium ion (LXIV) which picks up a hydride ion to give 3-amino-2-methylbenzyl alcohol. Sorkin, Krähenbühl and Erlenmeyer[27] have reported the preparation of the latter alcohol as an oil from 3-nitro-2-methylbenzoic acid (LXV) by formation of the acid chloride (LXVI) followed by conversion to the thiobenzyl ester (LXVII) and reduction with Raney nickel.



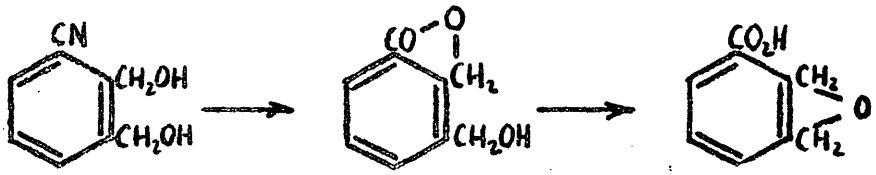
(LXV) (LXVI) (LXVII)

Thus to confirm structure (LXII) for the compound $C_8H_{11}ON$ it was necessary to synthesise 3-amino-2-methylbenzylalcohol by an unambiguous route. This has been accomplished by catalytically reducing the methyl ester of (LXV) and treating the resultant methyl 3-amino-2-methylbenzoate (LXVIII) with lithium aluminium hydride to give the compound $C_8H_{11}ON$ (LXII).



(LXVIII) (LXII)

We must assume that the product of Erlenmeyer and his co-workers was insufficiently purified. Conversion of the amine (LXI) to 3-cyanophthalyl alcohol (LXIX) followed by alkaline hydrolysis gave a low yield of an acidic product $C_9H_8O_3$ which we regard as phthalan-4-carboxylic acid (LX). The acid has presumably been formed by the base-catalysed isomerisation of the initially formed 4-hydroxymethylphthalide (LXX).



(LXIX) (LXX) (LX)

4-Hydroxymethylphthalide was not obtained from the uncrystallisable neutral fraction of the alkaline hydrolysis.

Brown and Newbold[1] have shown that 5:6-dimethoxyphthalan-4-carboxylic acid is isomerised to 6:7-dimethoxy-4-hydroxymethylpththalide on heating with mineral acid. A similar

acid treatment of phthalan-4-carboxylic acid gave a solid neutral product which was not obtained pure but whose infrared absorption spectrum in nujol showed hydroxyl (3390 cm.$^{-1}$) and phthalide carbonyl (1770 cm.$^{-1}$) peaks indicating that it was crude 4-hydroxymethylphthalide.

EXPERIMENTAL.

All melting points are uncorrected. Ultraviolet absorption spectra were determined in ethanol solution.

Synthesis of Gladiolic Acid Hydrate Triacetate.

5-Methoxy-6-methylphthalan-4-carboxylic Acid. - 4-Hydroxymethyl-7-methoxy-6-methylphthalide (600 mg.) was heated under reflux for 7 hr. with a solution of methanolic sodium methoxide from sodium (328 mg.) and methanol (15 c.c.). The cooled solution was diluted with water, made acid to Congo red with hydrochloric acid (d,1.15) and extracted with chloroform (3 x 30 c.c.). The combined extract was washed with aqueous sodium hydrogen carbonate (3 x 80 c.c.; 10%), water and dried (Na_2SO_4); evaporation of the chloroform gave starting material (34 mg.). The alkaline washings were acidified (Congo red) with hydrochloric acid (d,1.15) and extracted with chloroform (3 x 40 c.c.). The combined extract was washed with water, dried (Na_2SO_4) and evaporated to dryness under reduced pressure. The residue crystallised from water to give 5-methoxy-6-methylphthalan-4-carboxylic acid (560 mg.) as needles, m.p. 131-132° (Found: C,63.8; H,5.6%; equivalent, 207. $C_{11}H_{12}O_4$ requires C,63.45; H,5.8%; equivalent, 208). It had pK_a 3.13. Light absorption: Max. at 2140 (ε = 16,000) and 2970 (ε = 3,000), and inflexion at 2350 Å. (ε = 6,000).

Gladiolic Acid Hydrate Triacetate. - 5-Methoxy-6-methylphthalan-4-carboxylic acid (200 mg.) in dry benzene (12 c.c.)

and dry carbon tetrachloride (12 c.c.) was heated under reflux on the steam-bath, with irradiation from an adjacent 60 w. lamp, for 15 min. with N-bromosuccinimide (385 mg.). The cooled solution was filtered from succinimide and the filtrate evaporated under reduced pressure to yield a brown oil which was heated on the steam-bath with water (10 c.c.) for 1½ hr. with frequent shaking. The cooled solution was extracted with chloroform (3 x 25 c.c.) and the combined extract was washed with aqueous sodium hydrogen carbonate (3 x 50 c.c.; 10%). Acidification of the combined alkaline extract with hydrochloric acid followed by extraction with chloroform (3 x 50 c.c.) and evaporation of the dried (Na_2SO_4) chloroform extract under reduced pressure gave crude gladiolic acid. The hydrate triacetate was prepared according to the directions of Brown and Newbold.[5] It separated from aqueous ethanol as needles (39 mg.), m.p. 130-131°. The m.p. was undepressed on admixture with an authentic specimen having the same m.p. (lit.,[5] m.p. 131-132°). (Found: C,55.6; H,5.3. Calculated for $C_{17}H_{18}O_9$: C,55.7; H,4.95%), light absorption: Max. at 2140 (ε = 37,000) and 2980 (ε = 3,400), and inflexion at 2320 Å. (ε = 9,000).

Synthesis of Indane Derivatives.

7-Ethoxy-6-methoxyphthalide. - (a) Meconin (3.0 g.) was heated under reflux with ethyl sodiomalonate in ethanol [from ethyl malonate (4.96 g., 2 mols.), sodium (0.71 g.),

and ethanol (25 c.c.)] for 17 hr. The solution was concentrated under reduced pressure to 12 c.c., an equal volume of water added, and the precipitated oil (2.4 g.), which rapidly solidified, was separated. Crystallisation of the solid from aqueous ethanol gave 7-ethoxy-6-methoxyphthalide (2.0 g.) as needles, m.p. 66-67.5° (Found: C,63.9; H,5.9. Calculated for $C_{11}H_{12}O_4$: C,63.45; H,5.8%), light absorption: Max. at 2150 (ε = 27,000) and 3090 (ε = 4,500), and inflexion at 2400 Å. (ε = 7,200); infrared bands in nujol at 1754 (C=O stretching frequency) and 1592 $cm.^{-1}$ (benzene ring). The same product (2.1 g. from 3.0 g. meconin), m.p. and mixed m.p. 66-67°, was obtained by using ethyl acetoacetate (4.03 g.)in place of ethyl malonate. The m.p. of the compound was undepressed when mixed with 7-ethoxy--6-methoxyphthalide, m.p. 65.5-66° (lit.,[18] m.p. 68-69°) prepared by ethylation of 7-hydroxy-6-methoxyphthalide according to the method of Rodionow et al.[18] 7-Hydroxy-6--methoxyphthalide had light absorption maxima at 2190 (ε = 23,000) and 3120 (ε = 3,800), and inflexion at 2350-2400 Å. (ε = 6,500). It also showed strong infrared bands at 1751 (in CCl_4) and 1739 $cm.^{-1}$ (in $CHCl_3$), characteristic of the hydrogen bonded-carbonyl stretching vibration[20] (we are indebted to Mr. J. F. Grove and Dr. L. A. Duncanson for this determination). 7-Hydroxy-6-methylphthalide gave an indigo-blue colour with ferric chloride in aqueous ethanol solution.

(b) A solution of meconin (3.0 g.) in ethanol (25 c.c.) containing sodium ethoxide from sodium (0.71 g.) was heated under reflux for 20 hr. The solution was concentrated under reduced pressure, diluted with water, and extracted with ether: evaporation of the ether gave a negligible residue. The aqueous phase was acidified (Congo red) with hydrochloric acid and extracted with ether. The dried (Na_2SO_4) ethereal extract on evaporation gave an amber oil (2.95 g.) which was extracted with boiling light petroleum (b.p. 60-80°) (5 x 30 c.c.). The combined extract was concentrated and the solid which slowly separated was repeatedly crystallised from light petroleum (b.p. 60-80°) to give 7-ethoxy-6--methoxyphthalide (1.0 g.) as needles, m.p. 66-67.5°. The m.p. was undepressed when the compound was mixed with 7-ethoxy-6-methoxyphthalide prepared from 7-hydroxy-6-methoxyphthalide (Found: C, 63.8; H, 6.0%). 7-Ethoxy-6-methoxyphthalide was unaffected by prolonged treatment under reflux with methanolic sodium methoxide.

4-Chloromethyl-7-ethoxy-6-methoxyphthalide. - (a) (cf. Manske and Ledingham, Canad. J. Res., 1944, 22, B, 115). 2-Ethoxy-3-methoxybenzoic acid (0.5 g.) was heated under reflux for 35 min. with aqueous formaldehyde (1.25 c.c.; 40%) and hydrochloric acid (2 c.c.; d, 1.15). The mixture was diluted with water, cooled and extracted with chloroform. The extract was washed with aqueous sodium hydrogen carbonate,

water and dried (Na_2SO_4); evaporation of the chloroform under reduced pressure gave a light-brown oil which solidified. The solid was purified by filtration in benzene solution through a bed of alumina (3 x 1.5 cm.). The filtrate and benzene washings were evaporated under reduced pressure to give a clear gum which rapidly solidified; crystallisation of the solid from benzene-light petroleum (b.p. 40-60°) gave 4-chloromethyl-7-ethoxy-6-methoxyphthalide as felted needles, m.p. 126.5-127° (lit. m.p. 130°).

(b) 7-Ethoxy-6-methoxyphthalide (250 mg.) was heated under reflux for 45 min. with aqueous formaldehyde (2.57 c.c.; 40%) and hydrochloric acid (4.29 c.c.; d,1.15). Extraction of the cooled, diluted mixture with chloroform yielded a gum (0.28 g.) which rapidly solidified. A solution of the solid in benzene (25 c.c.) was passed through a column of alumina (3 x 1.5 cm.) and the eluate and benzene washings (125 c.c.) were evaporated to dryness. The residue was five times crystallised from benzene-light petroleum (b.p. 60-80°) and sublimed at 100°/10^{-3} mm., to give 4-chloromethyl-7-ethoxy-6-methoxyphthalide, m.p. 128-129.5°. The compound did not depress the m.p. when mixed with a sample of the preparation from (a) above (Found: C,56.5; H,5.25. Calculated for $C_{12}H_{13}O_4Cl$: C,56.15; H,5.1%).

2-Ethoxy-6-hydroxymethyl-3-methoxybenzhydrazide. - 7-Ethoxy-6-methoxyphthalide (150 mg.) was heated under reflux with 90% hydrazine hydrate solution (5 c.c.) and ethanol (10 c.c.) for 4 hr. The mixture was concentrated under reduced pressure, diluted with water, and extracted with chloroform (3 x 10 c.c.). The combined extract was washed with water, dried (Na_2SO_4) and evaporated to dryness under reduced pressure. The white solid residue (44.8 mg.) was recrystallised from benzene-light petroleum (b.p. 60-80°) to give 2-ethoxy-6-hydroxymethyl-3-methoxybenzhydrazide as needles, m.p. 149.5-150.5° (Found: C,54.9; H,6.6. $C_{11}H_{16}O_4N_2$ requires C,55.0; H,6.7%), light absorption: Max. at 2100 (ε = 23,000) and 2840 (ε = 2,500), and inflexions at 2325 (ε = 13,000) and 3050 Å. (ε = 1,600).

3-Ethoxy-4-methoxyphthalyl alcohol. - 7-Ethoxy-6-methoxyphthalide (250 mg.) in dry ether (30 c.c.) was added dropwise to a stirred, refluxing part-solution of lithium aluminium hydride (0.1 g.) in ether (100 c.c.) and refluxing continued for 4 hr. The cooled mixture was treated with crushed ice and made alkaline with aqueous sodium hydroxide (15 c.c.; 10%). The ethereal layer was separated and the aqueous phase extracted with ether (3 x 25 c.c.). The combined ethereal solutions were washed with water, dried (Na_2SO_4) and evaporated to give a clear gum (123 mg.) which

solidified on standing. Crystallisation from benzene-light petroleum (b.p. 40-60°) gave 3-ethoxy-4-methoxyphthalyl alcohol as felted needles, m.p. 64-65° depressed to 50-59° when mixed with starting material (Found: C,62.2; H,7.7. $C_{11}H_{16}O_4$ requires C,62.25; H,7.6%), light absorption: Max. at 2090 (ε =18,500), 2240 (ε = 7,800) and 2820 Å. (ε = 2,000).

Dimethyl α-(1:3-Dihydro-6:7-dimethoxy-1-oxo-4-benzo[c] furylmethyl)malonate. - A solution of 4-chloromethylmeconin (4.85 g.) in warm methanol was added in one portion to methanolic methyl sodiomalonate prepared from methyl malonate (2.64 g.), methanol (25 c.c.) and sodium (0.46 g.). The solution was heated under reflux for 3 hr., rapid separation of sodium chloride being observed. The mixture was concentrated under reduced pressure, and the residue diluted with water (25 c.c.) and extracted with chloroform (3 x 30 c.c.). The combined extracts were washed with aqueous sodium hydrogen carbonate (2 x 30 c.c.; 10%) (combined extract A), then water, and dried (Na_2SO_4). Evaporation of the chloroform under reduced pressure gave a clear yellow gum (5.72 g.) which partially solidified. The crystals (solid B) were separated by the addition of benzene (25 c.c.) and filtration, the filtrate being percolated down a column of Grade II alumina (2 x 6 cm.). The column was eluted with benzene (50 c.c.) and the combined eluate evaporated to

dryness under reduced pressure. The residual gum was dissolved in methanol (36 c.c.) concentrated to 12 c.c., and kept at 0°. The crystalline solid which gradually separated was extracted with warm methanol (35 c.c.) and filtered (solid C). The filtrate plus washings were concentrated and, on cooling, they deposited crystals, m.p. 122-124° (1.42 g.), which separated from methanol to give dimethyl α-(1:3-dihydro-6:7-dimethoxy-1-oxo-4-benzo[C] furylmethyl)malonate as prismatic needles, m.p. 123-125°, or blades from benzene-light petroleum (b.p. 60-80°) (Found: C,57.0; H,5.4. $C_{18}H_{18}O_8$ requires C,56.8; H,5.4%). The compound sublimes at 130°/10^{-4} mm. (bath temperature). Light absorption: Max. at 2100 (ε = 48,000) and 3110 Å. (ε = 5,600).

Dimethyl αα-Di-(1:3-Dihydro-6:7-dimethoxy-1-oxo-4--benzo[C]furylmethyl)malonate. - Solids B (195 mg.; m.p. 186-191°) and C (182 mg.; m.p. 192-193°) from the preceeding experiment were combined and crystallised from benzene to give dimethyl αα-di-(1:3-dihydro-6:7-dimethoxy-1-oxo-4--benzo[C]furylmethyl)malonate (340 mg.) as needles, m.p. 197-198° (Found: C,59.5, 59.8; H,5.5, 5.2; OMe,34.3. $C_{27}H_{28}O_{12}$ requires C,59.55; H,5.2; 6OMe,34.2%).

Diethyl-α-(1:3-Dihydro-6:7-dimethoxy-1-oxo-4-benzo[C] furylmethyl)malonate. (With Dr. John Blair) - The same molar proportions being used as in the reaction described

above with methyl malonate, 4-chloromethylmeconin (6.0 g.) was heated under reflux for 2 hr. with ethanolic ethyl sodiomalonate. The solution was concentrated under reduced pressure, diluted with water, and extracted with chloroform. Evaporation of the extract gave a neutral fraction (5.9 g.), m.p. 80-100°. The solid was dissolved in the minimum volume of hot benzene and the solid (solid D) which separated on cooling collected. Fractional crystallisation of the material in the filtrate gave diethyl α-(1:3-dihydro-6:7-dimethoxy-1--oxo-4-benzo[C]furylmethyl)malonate (1.1 g.) which separated from benzene-light petroleum (b.p. 60-80°) as needles, m.p. 114-115° (Found: C,58.9; H,6.1. $C_{18}H_{22}O_8$ requires C,59.0; H,6.05%), light absorption: Max. at 2080 (ε = 44,000) and 3100 Å (ε= 4,300). No crystalline material was obtained from the bicarbonate-soluble fraction (0.5 g.).

Diethyl αα-Di-(1:3-Dihydro-6:7-dimethoxy-1-oxo-4-benzo[C] furylmethyl)malonate. (With Dr. John Blair) - The solid D was crystallised from benzene to give the disubstituted malonate (1.5 g.) as needles, m.p. 168-169.5° (Found: C,61.1; H,5.6. $C_{29}H_{32}O_{12}$ requires C,60.8; H,5.6%).

αα-Di-(1:3-Dihydro-6:7-dimethoxy-1-oxo-4-benzo[C]furyl-methyl)malonic Acid. (With Dr. John Blair) - Dimethyl αα-di--(1:3-dihydro —6:7-dimethoxy-1-oxo-4-benzo[C]furylmethyl) malonate (50 mg.) was refluxed in a mixture of ethanol (2.5 c.c.) and aqueous sodium hydroxide (2.5 c.c.; 2N) for 2 hr.

The ethanol was removed by distillation and acidification of the aqueous solution (Congo Red) with hydrochloric acid gave αα-di-(1:3-dihydro-6:7-dimethoxy-1-oxo-4-benzo[C]furyl-methyl) malonic acid (35 mg.) which separated from aqueous acetone as needles, m.p. 228° (Found: C, 58.2; H, 4.8%; equivalent, 245. $C_{28}H_{24}O_{12}$ requires C, 58.1; H, 4.7%; equivalent, 258). Esterification with diazomethane gave back the dimethyl ester, m.p. and mixed m.p. 197-198°. The acid was also obtained by alkaline hydrolysis of the diethyl ester and it separated from aqueous acetone as needles, m.p. and mixed m.p. 228°.

α-(1:3-Dihydro-6:7-dimethoxy-1-oxo-4-benzo[C]furylmethyl) malonic Acid. - (a) Dimethyl α-(1:3-dihydro-6:7-dimethoxy-1-oxo-4-benzo[C]furylmethyl)malonate (100 mg.) was heated under reflux with aqueous sodium hydroxide (4 c.c.; 2N) for 1 hr. The cooled solution was acidified (Congo red) with hydrochloric acid (d, 1.15) and the crystalline precipitate separated. Crystallisation from water gave α-(1:3-dihydro-6:7-dimethoxy-1-oxo-4-benzo[C]furylmethyl)malonic acid (80 mg.) as plates, m.p. 153-155° (decomp.) (Found: C, 54.1; H, 4.9%; equivalent, 165. $C_{14}H_{14}O_8$ requires C, 54.2; H, 4.55%; equivalent, 155), light absorption: Max. at 2100 (ε = 30,000) and 3130 Å. (ε = 4,450). (b) By the same method, diethyl α-(1:3-dihydro-6:7-dimethoxy-1-oxo-4-benzo[C]furylmethyl)malonate gave the acid as plates (from water), m.p. 153-155° (decomp.) alone or mixed with

preparation (a).

Esterification of a methanolic solution of the acid with an ethereal solution of diazomethane, followed by evaporation of the excess ether and methanol and recrystallisation of the residue from methanol gave dimethyl α-(1:3-dihydro-6:7-dimethoxy-1-oxo-4-benzo[C]furylmethyl)malonate as prismatic needles, m.p. and mixed m.p. 123-125°.

β-(1:3-Dihydro-6:7-dimethoxy-1-oxo-4-benzo[C]furyl) propionic Acid. - α-(1:3-Dihydro-6:7-dimethoxy-1-oxo-4-benzo [C]furylmethyl)malonic acid (100 mg.) was heated at 190-200° (bath temperature) at atmospheric pressure until decarboxylation was complete and the residue sublimed at 170-180°/10^{-3} mm. (bath temperature), to give β-(1:3-dihydro-6:7-dimethoxy-1-oxo-4-benzo[C]furyl)propionic acid (70 mg.) which separated from methanol as flat prisms, m.p. 166-168° (Found: C,58.5; H,5.3%; equivalent, 264. $C_{13}H_{14}O_6$ requires C,58.6; H,5.3%; equivalent, 266), light absorption: Max. at 2100 (ε = 33,500) and 3120 Å. (ε = 4,750). The methyl ester, prepared by using ethereal diazomethane, separated from aqueous methanol as flat prisms, m.p. 93-94° (Found: C,60.15; H,6.1. $C_{14}H_{16}O_6$ requires C,60.0; H,5.75%), light absorption: Max. at 2130 (ε = 30,000) and 3120 Å (ε = 4,600).

5:6-Dimethoxy-2:2-di(methoxycarbonyl)indane-4-carboxylic Acid. - (a) The sodium hydrogen carbonate extract A (above) was acidified (Congo red) with hydrochloric acid

(d,1.15) and extracted with chloroform (3 x 50 c.c.). After being washed with water, the combined extracts were dried (Na_2SO_4) and evaporated under reduced pressure to a light yellow gum (750 mg.) which was triturated with cold methanol (5 c.c.), and the resulting solid separated. Crystallisation of the solid from aqueous methanol gave 5:6-dimethoxy-2:2-di(methoxycarbonyl)indane-4-carboxylic acid (300 mg.) as blades, m.p. 131-132° (Found: C,57.2; H,5.5%; equivalent, 337.5. $C_{16}H_{18}O_8$ requires C,56.8; H,5.4%; equivalent, 338), light absorption: Max. at 2110 (ε = 25,700) and 2980 Å. (ε = 4,200).

(b) A solution of dimethyl α-(1:3-dihydro-6:7-dimethoxy-1-oxo-4-benzo[c]furylmethyl)malonate (338 mg.) in methanol (10 c.c.) containing sodium methoxide from sodium (23 mg., 1 equivalent) was heated under reflux for 8 hr. The solution was evaporated under reduced pressure, the residue partially dissolved in water (15 c.c.) and the mixture extracted with chloroform (25 c.c.). The aqueous phase was acidified (Congo red) with hydrochloric acid (d,1.15) and the resultant precipitate extracted with chloroform (3 x 25 c.c.). The combined extract (extract E) was washed with aqueous sodium hydrogen carbonate (2 x 40 c.c.) and the alkaline washings acidified (Congo red) with hydrochloric acid. Extraction with chloroform gave 5:6-dimethoxy-2:2-di(methoxycarbonyl) indane-4-carboxylic acid (190 mg.) which separated from

aqueous methanol as blades, m.p. 130-131.5° alone or mixed with preparation (a). Evaporation of extract E gave unchanged starting material (100 mg.) which separated from methanol as prismatic needles, m.p. and mixed m.p. 121-123°.

5:6-Dimethoxy-2-methoxycarbonylindane-2:4-dicarboxylic Acid. - A solution of dimethyl α-(1:3-dihydro-6:7-dimethoxy-1-oxo-4-benzo[c]furylmethyl)malonate (1.0 g.) in methanol (25 c.c.) containing sodium methoxide from sodium (0.55 g.), was refluxed for 9 hr., concentrated under reduced pressure and diluted with water. The acid fraction of the product was isolated through chloroform and sodium hydrogen carbonate; it was obtained as an amber gum (900 mg.) which solidified. Trituration of the solid with dry ether and filtration from a trace of insoluble material followed by concentration of the filtrate and treatment with light petroleum (b.p. 40-60°) gave a solid, two crystallisations of which from ether-light petroleum (b.p. 40-60°) gave 5:6-dimethoxy-2-methoxycarbonylindane-2:4-dicarboxylic acid (500 mg.) as prismatic needles, m.p. 163-165° (Found: C, 55.85; H, 5.2%; equivalent, 168. $C_{15}H_{16}O_8$ requires C, 55.55; H, 5.0%; equivalent, 162), light absorption: Max. at 2090 (ε = 19,000) and 2990 Å. (ε = 3,600).

5:6-Dimethoxyindane-2:2:4-tricarboxylic Acid. - (a) 5:6-Dimethoxy-2:2-di(methoxycarbonyl)indane-4-carboxylic acid (250 mg.) was heated on the steam-bath for 1 hr. with aqueous sodium hydroxide (5 c.c.; 2N). The cooled solution was made

acid (Congo red) with hydrochloric acid (d,1.15) and the precipitate collected. Crystallisation of the precipitate from water gave 5:6-dimethoxyindane-2:2:4-tricarboxylic acid (190 mg.) as prismatic needles, m.p. 185-187° (decomp.) (Found: C,54.5; H,4.8%; equivalent, 103. $C_{14}H_{14}O_8$ requires C,54.2; H,4.55%; equivalent, 103), light absorption: Max at 2080 (ε = 25,800) and 2975 Å. (ε = 3,900). The same acid, m.p. and mixed m.p. 185-187° (decomp.) was obtained by hydrolysis of 5:6-dimethoxy-2-methoxycarbonylindane-2:4-dicarboxylic acid.

(b) (With Dr. John Blair). - Diethyl α-(1:3-dihydro-6:7-dimethoxy-1-oxo-4-benzo[c]furylmethyl)malonate (1.25 g.) was added to a warm solution of sodium ethoxide in dry ethanol (25 c.c.) [from sodium (0.7 g.)]. The yellow solution was heated under reflux for 8 hr., concentrated to small bulk under reduced pressure, diluted with water and acidified (Congo red) with hydrochloric acid (d,1.15). The solution was extracted with chloroform and the chloroform extract washed with aqueous sodium hydrogen carbonate. The acidic fraction was isolated from the alkaline washings (by acidification and extraction with chloroform) as a clear, light-brown gum (1.0 g.). Hydrolysis of the gum, which did not solidify, with alkali as described in (a) gave 5:6-dimethoxyindane-2:2:4-tricarboxylic acid (0.55 g.), which crystallised from water as prismatic needles, m.p. 185-187°

alone or mixed with preparation (a) (Found: C,54.4; H,4.7%).

Methyl 5:6-Dimethoxyindane-2:2:4-tricarboxylate. - 5:6-Dimethoxy-2:2-di(methoxycarbonyl)indane-4-carboxylic acid (100 mg.) in methanol (5 c.c.) was treated with excess of ethereal diazomethane and kept overnight. Removal of solvent and crystallisation of the residue from benzene-light petroleum (b.p. 40-60°) gave methyl 5:6-dimethoxyindane-2:2:4-tricarboxylate as prisms, m.p. 78.5-80.5° (Found: C,58.3; H,6.0. $C_{17}H_{20}O_8$ requires C,57.95; H,5.7%), light absorption: Max. at 2080 (ε = 21,500) and 2990 Å. (ε = 4,200). The same ester, m.p. and mixed m.p. 78.5-80.5°, was obtained by the action of diazomethane on 5:6-dimethoxyindane-2:2:4-tricarboxylic or 5:6-dimethoxy-2-methoxycarbonylindane-2:4-dicarboxylic acid.

5:6-Dimethoxyindane-2:4-dicarboxylic Acid. - 5:6-Dimethoxyindane-2:2:4-tricarboxylic acid (100 mg.) was heated under atmospheric pressure at 200-210° (bath temperature) on the oil-bath until no further evolution of carbon dioxide took place. Sublimation of the residue at 160°/10^{-4} mm., followed by crystallisation from ethyl acetate-light petroleum (b.p. 40-60°) gave 5:6-dimethoxyindane-2:4-dicarboxylic acid (60 mg.) as prisms, m.p. 153.5-155° (Found: C,59.1; H,5.5%; equivalent, 131. $C_{13}H_{14}O_6$ requires C,58.6; H,5.3%; equivalent 133), light absorption: Max. at 2100 (ε = 23,000) and 2960 Å. (ε = 3,900).

Synthesis of Phthalan-4-carboxylic Acid.

2-Cyano-6-nitrotoluene. - (cf. Sorkin, Krähenbühl and Erlenmeyer, Helv. Chim. Acta, 1948, 31, 65). 2-Amino-6-nitrotoluene (5 g.) prepared from 2:6-dinitrotoluene according to the method of Brady and Taylor (J. Chem. Soc., 1920, 876), was dissolved in hot hydrochloric acid (7.5 c.c.; d,1.15) and water (75 c.c.) and cooled below 5°. Fine needle crystals of the amine hydrochloride separated and the mixture was diazotised with a cold solution of sodium nitrite (16.5 c.c.; 2N). Excess nitrous acid was destroyed by the addition of urea and the filtered solution was basified to pH6 by the addition of sodium carbonate. The solution was added portion-wise, with shaking, to a solution of copper cyanide at 60°, prepared by adding a solution of potassium cyanide (13.25 g.) in water (80 c.c.) gradually, with shaking, to a solution of copper sulphate (12 g.) in water (50 c.c.) and heating to 60° for a few minutes. After the addition was complete, the mixture was heated on the steam-bath at 50-60° for 1 hr. and then kept overnight at room-temperature. The brown crystalline product which separated was collected (5.13 g.), m.p. 66-68° (decomp.) (lit., m.p. 69.5°).

2-Methyl-3-nitrobenzoic Acid. - In our hands the acid hydrolysis of 2-cyano-6-nitrotoluene employed by Erlenmeyer et al. (loc. cit.), gave the benzoic acid in very poor yield.

The following method of alkaline hydrolysis proved to be much more efficient. 2-Cyano-6-nitrotoluene (1 g.) was heated under reflux with aqueous potassium hydroxide (50 c.c.; 10%) for 3 hr. On cooling and filtering from a slight residue, the filtrate was acidified (Congo red) with hydrochloric acid (d,1.15) and the precipitated 2-methyl-3-nitrobenzoic acid collected (0.85 g.), m.p. 180-182° (decomp.) (lit., m.p. 181°).

Methyl 2-Methyl-3-nitrobenzoate. - An ice-cooled solution of 2-methyl-3-nitrobenzoic acid (2.55 g.) in methanol (30 c.c.) was treated with excess of an ethereal solution of diazomethane and the reaction solution kept overnight. The filtered solution was evaporated and the residue crystallised from aqueous methanol (charcoal) to give methyl 2-methyl-3-nitrobenzoate (2.2 g.) as felted needles, m.p. 65-66° (Found: C,55.3; H,4.6. Calculated for $C_9H_9O_4N$: C,55.4; H,4.65%). The m.p. of the ester obtained[87] from the acid chloride is 66°.

Methyl 2-Methyl-3-aminobenzoate. - Methyl 2-methyl-3--nitrobenzoate (0.5 g.) in dry ethyl acetate (50 c.c.) was shaken with hydrogen at room temperature and pressure in the presence of Raney nickel catalyst (0.3 g.; W-2). Uptake of hydrogen was complete in 15 min. The reaction mixture was filtered through a bed of kieselguhr and the filtrate evaporated under reduced pressure to give methyl 2-methyl-3--aminobenzoate as a light yellow oil (0.4 g.)

Dimethyl 3-Aminophthalate. - (cf. Twiss and Heinzelmann, J. Org. Chem., 1950, 15, 496). A solution of dimethyl 3-nitrophthalate (3.12 g.) in dry ethyl acetate (150 c.c.) was shaken with hydrogen at room temperature and pressure in the presence of Raney nickel catalyst (1.2 g.; W-2). Uptake of hydrogen was initially very rapid and was complete in 2 hr. Filtration of the mixture through a bed of kieselguhr followed by evaporation of the solvent under reduced pressure gave dimethyl 3-aminophthalate (2.56 g.) as a clear oil.

3-Aminophthalyl Alcohol. - The above oil was dissolved in ether (100 c.c.) (The ethereal solution had a blue fluorescence) and added dropwise over 15 min. to a stirred refluxing solution of lithium aluminium hydride (2 g.) in ether (200 c.c.). After refluxing and stirring for 2½ hr., the reaction mixture was cooled to 0° and excess lithium aluminium hydride decomposed by the addition of crushed ice. The ethereal layer was separated and the aqueous layer extracted with ether (100 c.c.). The combined ethereal solutions were washed with water, dried (Na_2SO_4) and evaporated to give a solid residue (0.9 g.). Crystallisation from benzene-ethyl acetate-light petroleum (b.p. 60-80°) gave a top crop (0.36 g.; Mother liquor A) which, after several crystallisations from benzene, gave 3-aminophthalyl alcohol (0.25 g.; 14%) as needles, m.p. 96-97° (Found: C,63.0; H,6.7.

$C_8H_{11}O_2N$ requires C,62.7; H,7.2%), light absorption: Max. at 2080 (ε = 29,000), 2410 (ε= 6,000) and 2960 Å.(ε= 2,300). The triacetate was prepared by treating the alcohol (87 mg.) on the steam-bath for 3 hr. with acetic anhydride (2 c.c.) and pyridine (1 c.c.). The reaction solution was diluted with water and kept overnight. Extraction of the diluted solution with chloroform yielded the product (183 mg.) which separated from benzene-ether-light petroleum (b.p. 60-80°) as needles, m.p. 122-122.5° (Found: C,60.5; H,5.8. $C_{14}H_{17}O_5N$ requires C,60.2; H,6.1%), light absorption: Max. at 2075 (ε = 29,800) and 2340 (ε= 6,300), inflexion at 2700 Å. (ε = 1000).

When the reduction of dimethyl 3-aminophthalate was carried out with addition at 0° and the reaction mixture kept at 0° for 40 hr. the yield of pure 3-aminophthalyl alcohol was 16%.

3-Amino-2-methylbenzyl Alcohol. - (a) Mother liquor A above was concentrated to half bulk and treated with light petroleum (b.p. 60-80°). The crop of crystals which separated on cooling was dissolved in benzene (30 c.c.) and chromatographed on alumina (12 g.). Elution with benzene (100 c.c.) followed by a little benzene-ether mixture (1:1), and then ether (100 c.c.) gave, on evaporation of the combined eluates, a solid which was crystallised from benzene to give 3-amino-2-methylbenzyl alcohol as needles, m.p. 106-107°

(Found: C,70.1; H,7.8; N,10.3. $C_8H_{11}ON$ requires C,70.0; H,8.1; N,10.2%), light absorption: Max.at 2070 (ε = 26,800), 2355 (ε = 6,300) and 2900 Å (ε = 2,000). The <u>diacetate</u>, prepared by the action of acetic anhydride–pyridine on the steam-bath for 3 hr. and isolated through chloroform, separated from benzene-light petroleum (b.p. 60-80°) as felted needles, m.p. 147.5-148° (Found: C,65.6; H,6.4; N,6.7. $C_{12}H_{15}O_3N$ requires C,65.1; H,6.8; N,6.3%), light absorption: Max. at 2110 (ε = 21,500) and inflexion at 2310 Å (ε = 6,400). Further elution of the column with ether-methanol (1:1) and methanol gave 3-aminophthalyl alcohol, m.p. 96-97° (108 mg.).

(b) Methyl 2-methyl-3-aminobenzoate (1.53 g.) in ether (50 c.c.) was added over 20 min. to a stirred, refluxing solution of lithium aluminium hydride (0.8 g.) in ether (100 c.c.). After 2½ hr. refluxing and stirring, the reaction mixture was cooled to 0° and excess lithium aluminium hydride decomposed with crushed ice. The ethereal layer was separated and the aqueous layer extracted with ether (100 c.c.). Evaporation of the combined, dried (Na_2SO_4) ethereal solution gave a white solid residue (1.28 g.). Crystallisation of the residue from benzene gave 3-amino-2-methylbenzyl alcohol as needles, m.p. 106-106.5° alone or mixed with preparation (a). The infrared absorption spectra of preparations (a) and (b), determined in nujol, were identical.

(c) A solution of 3-aminophthalyl alcohol (0.4 g.) in tetrahydrofuran (30 c.c.) was added to a stirred, refluxing solution of lithium aluminium hydride (0.3 g.) in ether (75 c.c.). After 2½ hr. refluxing and stirring the excess lithium aluminium hydride was decomposed and the product isolated as described above. Chromatography as in (a) was used to separate the product into its two constituents. Elution of the column with benzene and ether gave 3-amino-2--methylbenzyl alcohol (246 mg.), crystallising from benzene as felted needles, m.p. 106-107.5° undepressed on mixing with preparation (a); elution with chloroform-methanol (1:19) gave unchanged 3-aminophthalyl alcohol (140 mg.) m.p. and mixed m.p. 93-97.5°.

Phthalan-4-carboxylic Acid. - A solution of 3-amino-phthalyl alcohol (1.0 g.) in hydrochloric acid (1.86 c.c.; d, 1.15) and water (18 c.c.) was diazotised with a solution of sodium nitrite (0.54 g.) in water (3 c.c.) at 0°. Meanwhile a solution of anhydrous nickel chloride (1.32 g.) and potassium cyanide (1.62 g.) in water (10 c.c.) was boiled for 2 min., cooled and neutralised with sodium carbonate (0.36 g.). To this solution, below 5°, was added the diazonium solution which had been neutralised with sodium carbonate. After keeping at room temperature overnight the reaction mixture was filtered (Residue B) and the filtrate extracted with

ether (3 x 50 c.c.). The combined ethereal extracts were washed with water, dried (Na_2SO_4) and evaporated to give a brown gum (222 mg.). Residue B was extracted with chloroform (2 x 50 c.c.) to yield a gum (246 mg.) on evaporation of the combined extract. The combined gums were heated under reflux with aqueous potassium hydroxide (25 c.c.; 10%) for 3 hr. The cooled solution was acidified (Congo red) with hydrochloric acid (d,1.15) and the resulting precipitate extracted with chloroform (3 x 25 c.c.). The combined extracts were washed with aqueous sodium hydrogen carbonate (2 x 50 c.c.; 10%), water and dried (Na_2SO_4). Evaporation of the extract to dryness under reduced pressure gave an intractible red gum (72 mg.). The alkaline washings were acidified (Congo red) with hydrochloric acid (d,1.15) and extracted with chloroform the dried (Na_2SO_4) extract, on evaporation under reduced pressure, gave a solid (114 mg.). Crystallisation of the solid from benzene (charcoal) gave phthalan-4-carboxylic acid as needles, m.p. 195.5-199° (Found: C,65.5; H,5.2. $C_9H_8O_3$ requires C,65.85; H,5.2%), light absorption: Max. at 2065 (ε = 19,600), 2300 (ε = 9,500) and 2850 Å. (ε = 2,200).

Attempted Preparation of 4-Hydroxymethylphthalide. - Phthalan-4-carboxylic acid (32 mg.) was heated under reflux for 3 hr. with hydrochloric acid (10 c.c.; 5N). The cooled solution was diluted with water (30 c.c.) and extracted with chloroform (3 x 40 c.c.). The combined extracts were washed

with aqueous sodium hydrogen carbonate (2 x 25 c.c.), water and dried (Na_2SO_4). Evaporation of the solvent under reduced pressure gave a partly solid material (17 mg.) which was sublimed at 70°/10^{-3} mm. to give a yellow solid, trituration of which with a little dry ether gave a residual white solid, m.p. 103-105.5° (2 mg.). The infrared absorption spectrum of the product in nujol shows bands at 3390 (hydroxyl stretching vibration) and 1770 $cm.^{-1}$ (phthalide carbonyl stretching vibration).

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